SCIENTIFIC AGRICULTURE

Vol. XVI MARCH, 1936 No. 7

# RASPBERRY NUTRITION

## II. Causes of Raspberry Failures in the Coastal Area of British Columbia

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[Received for publication January 10, 1936]

In a previous issue of Scientific Agriculture a paper appeared entitled *Raspberry Nutrition I* by the writer and J. J. Woods (*1*). It was reported in this article that the soils in the Fraser Valley of British Columbia were, in general, in a poor physical condition during the winter months. The most common cause for this poor physical condition was found to be due to the leaching of calcium from the soil by the winter rains. Fall applications of lime were recommended to remedy this loss. The winter rains, also were found to be responsible for tremendous leaching losses of other minerals and nitrates. Winter cover cropping was advocated as a remedy for this situation.

The typical British Columbia coastal seasonal trend of available nutrients in a fertile soil was found to run as follows: In March the nutrients are at a minimum. Between March and May they increase rapidly, reaching a maximum in May. During the period between May and July they decrease again and in July reach a point approaching the minimum. Sometime between July and September they increase once more, and in September a second maximum is reached. Following the high September concentration, a fairly rapid decline takes place, so that by November a low figure is reached again. This decline continues rapidly throughout the winter until the following March, when the minimum value prevails once more. The seasonal trend of available nutrients gives a very definite lead as to the most advantageous time for the application of fertilizers. The nitrogen curve may be used as an illustration.

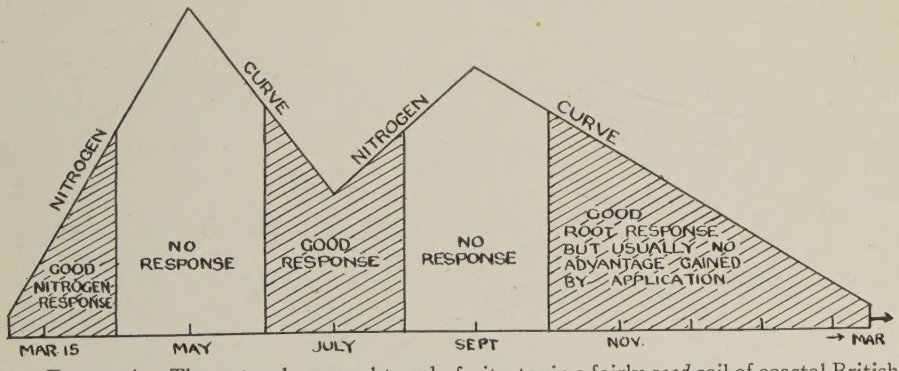


Figure 1. The natural seasonal trend of nitrates in a fairly *good* soil of coastal British Columbia. (The shaded portions show the time when a response can be obtained from an application of readily available nitrogen. For further explanation see text.)

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Figure 1 shows that just at the time when most of our crops are using nitrogen heavily, the natural supply has started to fall and needs "bolstering up" by a supply of artificial nitrogen. For crops making growth in the summer months—July and August—nitrates at least should be added at this time. This is especially true of lawns and pastures.

On the other hand, in most of our poorer soils a response can be obtained almost at any time with a nitrogen application. It should be especially supplied, in available form, where Figure I indicates no response on good soils.

The experimental plots reported on were less acid in summer than in winter. Plots growing cover crops were less acid than those without. For the first year all nutrients, especially nitrates, were lower in plots growing cover crops than those not growing them. Of the cover crops used, rye reduced the nutrients least, and clover the most.

From the analyses made, tables were drawn up for future reference. The tables showed the amounts of available nutrients necessary throughout the year in order to ensure healthy growth.

These tables have been used since by the writer to diagnose soil troubles from raspberry plantings where difficulties have been encountered, and also to evaluate soils for new plantings.

## TYPICAL CONDITIONS WHICH ARE FOUND RESPONSIBLE FOR POOR GROWTH AND LOW YIELDS IN COASTAL BRITISH COLUMBIA

*Nitrogen*

Probably the most common difficulty encountered is nitrogen shortage. Most soil samples sent in to this laboratory from raspberry plantings have shown a nitrate value far below that in our tables. Usually the plants have depleted the original supply and are still capable of utilizing more nitrates than are actually produced by the soil organisms. Such a situation means that the soil either must be given an application of nitrates, or be let lie fallow for some time (which latter is impossible when planted in raspberries). Once the nitrate supply has been lowered almost to the point of extinction, as is the case in many of our raspberry plantings, a single application of nitrogen will not bring it back. It may take two or three years of regular applications to supply the need and get the soil back to a satisfactory biological condition.

*Lime*

Lime is deficient in most plantings. Besides the fact that the plants actually need calcium, many other beneficial effects occur to the soil as a result of its application. It is advisable, however, to have a lime requirement test made of a soil before applying it.

*Phosphorus*

Many plantings giving low yields have shown a phosphorus deficiency. Here the problem is not so simple as it looks at first. It is true that most of our soils give low phosphorus tests, yet some giving low tests produce satisfactorily. This depends on the physical condition of the soil and its supplying power in regard to phosphorus. Some soils have a very low phosphorus content, but maintain it continuously throughout the year. Other soils show quite a high phosphorus content at some periods, but at others give almost a negative test. The former soil will maintain growth, the latter will not. Another difficulty encountered with phosphorus is in

the use of lime. Quite often a soil will give a low phosphorus test and at the same time show a lime deficit. Unfortunately, calcium tends to combine with phosphorus, yielding an insoluble compound. In such a case an application of lime will remedy the calcium deficit, but will create a phosphorus deficit, which is equally as bad as the original condition.

Phosphates should not be mixed with lime or applied at the time of, or just after liming. They should be applied preferably a year or more in advance of the lime. Acid phosphate and manure may be applied either before or after liming. Lime, especially the burnt hydrated forms, should not be mixed with manure.

*Potash*

Available potash runs low in most of our raspberry plantings. In some cases it is the main cause of difficulty. Certain low yielding plots have responded immediately to potash fertilizers. Others have not. With an application of any fertilizer, three things can happen:

1. The theoretical case which rarely, if ever, happens: the plant has available for it the extra amount of added material.

2. The plant gets more of the mineral than you actually add, due to changes taking place in the soil as a result of its application.

3. The plant gets little or none of the added mineral, because it reacts with the soil and becomes insoluble or unavailable, but it may get more of something else released from the soil in its place.

An interesting example of this last case, known as base exchange, was observed in the original experiments. Additions of sodium nitrate increased the available potash, even though no potash was added. The sodium remained in the soil complex, but replaced and made available to the plant an equivalent amount of potash which was otherwise present in the soil but unavailable. In effect, then, the sodium nitrate application was the equivalent of a potash fertilizing.

In cases where available potash is deficient and yet the soil does not respond to an ordinary application of it, either extremely large amounts must be added until the soil complex becomes saturated with potash, or other expedients may be tried. Sometimes an application of sodium nitrate temporarily may serve a double purpose of supplying nitrates and potash.

*Acidity* (Soil Reaction)

The acidity question is extremely important. Very many plantings showing troubles are very acid in reaction. The damage due to the acid itself is not often serious; however, secondary effects may be very much so. Some plots which give quite an acid reaction maintain good growth. Others which show the same acid reaction give very poor results. Availability of nutrients is very closely tied up with acidity. Some minerals become insoluble, i.e., unavailable, in acid soils; others become very soluble, i.e., available. In general the essential ones are rendered insoluble, while toxic ones become soluble.

Nitrate forming organisms will not work at all, or not efficiently, under too acid conditions.

Iron in small quantities is an essential for plants, but an overdose is toxic. Aluminum is essential in small traces only—even very small quantities are toxic. In some soils iron and aluminum come into solution in

sufficient quantities to cause toxicity under acid conditions, whereas in the same soils under neutral conditions they remain relatively insoluble and beneficial.

We have found available aluminum in sufficient quantities to cause severe injury to plants, present in some plantings, giving an acid soil reaction. Applications of lime are necessary to neutralize such soils and render the aluminum insoluble. The precautions mentioned under phosphorus, however, must be kept in mind.

*Unbalanced Nutrients*

Another cause of difficulty not infrequently met with is an unbalanced nutrient supply. One must have a fairly uniform supply of all salts rather than an excess of one and a deficiency or absence of another. Even if all elements are present in sufficient quantities for good growth, but one is present in a great excess over the others, trouble will ensue. The excess of any one element retards the normal entry into the plant of other minerals, so that starvation occurs amidst plenty. The importance of salt ratios and balances cannot be over-emphasized.

*Cover Crops*

Frequently we find growers who were going to plant cover crops immediately, and who would be ill advised to do so. An analysis of their soil shows it to be markedly deficient in certain minerals. While the value of cover crops cannot be over-emphasized, for the first year of their establishment they do reduce the available nutrients to a lower point than if the plot had been clean cultivated. It is true that those minerals are returned to the soil when the cover crops are turned in, but in the meantime they are competing with the crop. Before cover crops are planted, then, make sure that the soil has sufficient nutrients to carry it and the crop for the first year. If not, supplement any deficiencies with artificial fertilizers.

*"Wet Feet" and Root Injury*

Any factor which prevents good soil aeration causes a cessation of root growth and the probable death of the root. Excess moisture is one of the most usual ways in which poor soil aeration is brought about. The water itself does not hurt the root, but it usually becomes saturated with carbon dioxide and cuts off the air supply to the root. The more easily soils puddle, the more deficient is the air supply. Soils low in calcium puddle very easily.

In localities such as coastal British Columbia, where mild winters are prevalent, it is the natural condition for root growth to continue during the winter months. (*2*) Root injury due to excess moisture, then, is liable to occur even in winter because of winter root growth.

Injury to roots, due to poor soil aeration, may result from several causes. The most obvious cause is that lack of oxygen interferes with normal respiration. Respiration is an oxidation process whereby energy is released. If oxygen is deficient the roots are not provided with sufficient energy to carry on their normal activities, and die as a result.

Another point is that a new, actively growing and elongating root is white and succulent. As it ages or as freezing temperatures approach, it shrinks in size and becomes protected by a brown coating of varnish (suberin). In this protected stage frost does not injure it. In the unprotected stage even slight soil freezing temperatures will cause damage (*2*).

This varnish is excreted from the cells inside the root. When it reaches the surface it spreads out in a film over the root and as a result of coming in contact with the oxygen in the soil air it hardens similarly to any other quick drying varnish. (*3*). If, on the other hand, the soil is waterlogged or deficient in oxygen, this varnish will not harden and the root remains unprotected. This is the reason why winter injury to roots is usually severe in wet places. On well drained, well aerated soils, new roots are well suberized and protected against frost. In waterlogged, badly aerated soils, young roots have no protective covering.

A further cause of injury is frequently recorded, due to excessive soil moisture in spring, when top growth first starts. Plants which start out under excessive moisture conditions develop large leaves and initially make succulent growth (*4*). They develop a certain water requirement greatly in excess of the plant which starts out under conditions where moisture is not so abundant. If a dry season occurs it naturally follows that the plant having the greater water requirement is going to suffer much more than the one having the lesser water requirement.

Furthermore, as a result of waterlogged soils an acid condition arises and the difficulties enumerated under acidity are to be contended with.

The advantages of well drained soil at all times of the year are obvious.

## CONCLUSION

The above mentioned causes of raspberry failures are not theoretical probabilities. They are all taken from records of actual cases investigated from the viewpoint of the plant nutritionist. While such factors as bacterial blight, virus disease, fungus and insect attacks, and depredations by moles, have been previously recorded by other workers as responsible in certain specific cases for raspberry failures, on the other hand, a very large number of cases previously have not been satisfactorily diagnosed.

The writer considers that the nutritional disorders mentioned in this article are responsible for a very great number, if not most, of the difficulties encountered in the small fruit industry of British Columbia.

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## Résumé

**Nutrition du framboisier par G. Howell Harris, Université de la Colombie-Britannique, Vancouver, C.-B.**

Les causes de la mauvaise pousse du framboisier décrites dans cet article ne sont pas des probabilités théoriques. Elles sont toutes extraites des dossiers de cas actuels, étudiés au point de vue de la nutrition. La responsabilité de certains facteurs dans ces insuccès, comme la maladie bactérienne, la maladie à virus, les maladies cryptogamiques des insectes, les déprédations par les taupes, ont déjà été déterminées par d'autres investigateurs, mais il y a un très grand nombre d'autres cas dont il n'a pas été fait de diagnostic satisfaisant. L'auteur considère que les troubles de nutrition mentionnés dans cet article sont la cause d'un très grand nombre, sinon de la plupart des difficultés que présente l'industrie de la culture des petits fruits en Colombie-Britannique.

# CONTROL OF LATE BLIGHT OF CELERY[1]

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[Received for publication March 7, 1936]

## INTRODUCTION

The practice of applying fungicides to celery, for the control of leaf spots or blights, has been a recommended procedure for years, yet many growers still suffer considerable losses from these diseases. The object of the present investigations has been to compare the effectiveness of certain fungicides in controlling late blight of celery under conditions prevailing in the Niagara peninsula.

## OCCURRENCE OF BLIGHTS

Although both early blight (*Cercospora Apii* Fr.) and late blight (*Septoria* spp.) are common in the celery growing districts of Ontario, the latter is responsible for the greater damage. Cochran (2) has described two different leaf spots caused by distinct species of the genus *Septoria*. Both have been observed in the Niagara peninsula, but the "small-spot" type caused by *Septoria Apii-graveolentis* Dorogin (Figure 1) is more preva-

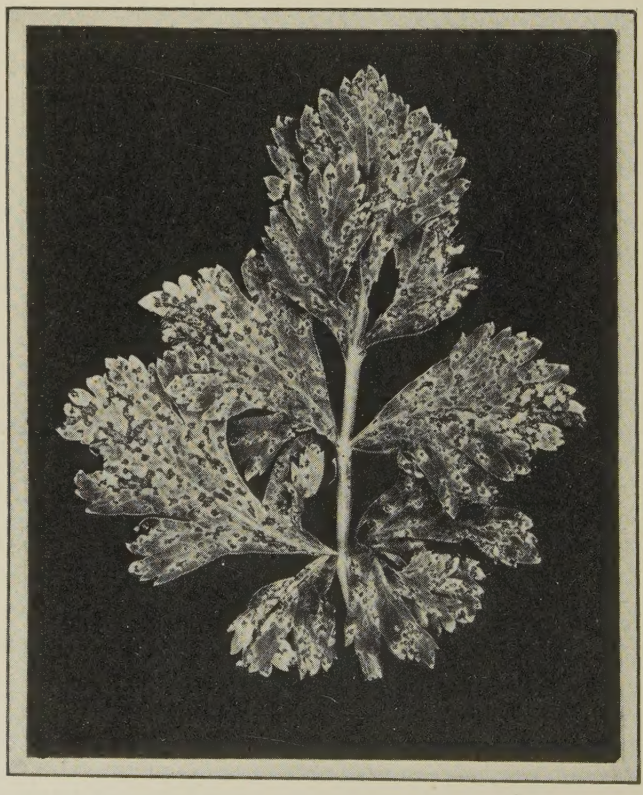

FIGURE 1. A celery leaf severely infected by *Septoria Apii-graveolentis* Dorogin.

[1] Contribution No. 449 from Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada.

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lent and more serious than the "large-spot" type caused by *Septoria Apii* Chester. In the ensuing experiments only the "small-spot" type of late blight is considered, since neither of the other types appeared to an appreciable extent in any of the plots.

FIGURE 2.—Two plots set with similar plants and growing immediately adjacent to one another. The upper received no fungicide while the lower was sprayed with Burgundy mixture every ten days. This comparison shows the severe damage that can be caused by late blight.

While growers generally concede that the application of fungicides to celery is a necessity, too often the matter is considered of secondary importance, with the result that the disease becomes established before steps are taken to prevent it. This delay greatly increases the difficulty of obtaining adequate control.

## METHODS AND MATERIALS

The present investigations have been carried out under field conditions for the past five years, with minor annual changes, some of which were necessitated by the location of the plots, while others were suggested by previous results. In the first year of the investigation when only natural sources of infection were relied upon, no significant differences in amount of disease occurred in the plots. This condition was remedied in the following years by interspersing artificially-infected plants at regular intervals throughout the rows. The plots were so arranged as to minimize the drift of fungicides from one to another.

The Paris Golden variety was used throughout the experiments. The seed was sown in the greenhouse during the last week of February, the seedlings transplanted into flats early in April, and the young plants set out in the field the latter part of May or the beginning of June, when they were from six to eight inches in height. The individual plots were set with healthy plants in rows eighteen inches apart, with six inches between the plants in the rows. In each plot, except the check, an equal number of vacant spaces was left to be planted with diseased plants after the first application of the fungicide. In addition to the check plots comprising entirely healthy stock, control plots containing healthy and diseased plants not subjected to treatment, were set out to determine the spread of the disease due to artificial inoculation, as well as its natural occurrence in the field.

Although a review of the literature shows that investigators generally are agreed that copper compounds, either in the form of sprays or dusts, are the best fungicides to use for controlling celery blights (*1*, *3*, *4*, *5*), it was thought advisable to test a few of the more common sulphur compounds to determine their effect under local conditions.

It is conceded that maximum control can only be effected by keeping the new growth adequately covered with fungicides, which generally requires application at least once a week, or even more frequently, depending on the growth-rate of the host. The slightly longer interval of ten days between applications was chosen in these investigations to accentuate the relative effectiveness of the fungicides, and to eliminate the possibility of a less effective compound being applied at intervals short enough to produce results as satisfactory as those obtained with a superior material. In 1934, in order to subject the chemicals to a still more rigid test, they were also applied to duplicate series of plots every twenty days. The dusts were applied early in the morning while the dew was still on the plants, and the liquids later the same day. The dry materials were applied with a hand duster, and the liquids, in 1932, with a small hand sprayer, and, in 1933 to 1935, with a hand-pump barrel machine developing much higher pressure.

All treated plots were sprayed or dusted at regular intervals throughout the growing season, commencing shortly after the plants were set in the

field, and continuing until ten days prior to the recording of the final observations. From nine to twelve applications were made annually, depending on the length of the growing season.

Observations on the amount of disease present were made three times during the first four seasons; the first, one month after the initial application of fungicide; the second, in July or August, depending on the rate of increase of the disease, and the last, ten days after the final application of the fungicide. In 1935, the intermediate reading was omitted. The severity of the disease recorded for each plant, except those artificially infected and interspersed in the plots to provide inoculum, was divided into the following five categories:

Nil—no lesions visible on any of the leaves.

Trace—fewer than ten infected areas on a plant.

Slight—several infected areas on a number of leaves.

Moderate—a general infection of the plant without killing of leaves.

Severe—a severe, general infection with killing of the older leaves.

While it is admitted that under this arbitrary arrangement, borderline cases were sometimes difficult to classify, the use of these five categories has made it possible to obtain a reasonably accurate conception of the prevalence of the disease in individual plots.

The following list enumerates the fungicides that were tested in the course of the present investigations, and the number of seasons each was used.

| | *Years* |
|---|---|
| [1]Burgundy mixture, 4-5-40 | 4 |
| Burgundy plus calcium caseinate, 1 lb. to 40 gals. | 1 |
| Burgundy mixture, 2-2½-40 | 1 |
| Bordeaux mixture, 4-6-40 | 2 |
| Bordeaux mixture, 4-4-40 | 2 |
| Bordeaux mixture, 4-6-40, plus calcium caseinate, 1 lb. to 40 gals. | 2 |
| Bordeaux mixture, 4-6-40, plus calcium arsenate, ½ lb. to 40 gals. | 1 |
| Bordeaux mixture, 4-4-40, plus lethalate, 1 oz. to 40 gals. | 2 |
| Bordeaux mixture, 2-2-40 | 1 |
| Bordeaux mixture, 2-2-40, plus lethalate, 1 oz. to 40 gals. | 1 |
| Copper-lime dust, 20-80 | 4 |
| Copper carbonate-lime dust, 20-80 | 1 |
| Copper carbonate-lime dust, 50-50 | 1 |
| Colloidal copper | 1 |
| Colloidal copper plus calcium caseinate, 1 lb. to 40 gals. | 1 |
| Lime sulphur, 1-50 | 2 |
| Sulphur-lime dust, 90-10 | 2 |
| Kolofog plus lime, 4-3-40 | 2 |
| Koloform, 8.5 lbs. to 40 gals. | 1 |

Not all the compounds were used in any one year as the graph (Figure 3) shows, but as the experiments progressed, materials which exhibited weak fungicidal properties were discarded and replaced by more promising ones.

[1] A fungicide prepared in the same way as Bordeaux mixture, but using sodium carbonate (sal soda) instead of lime.

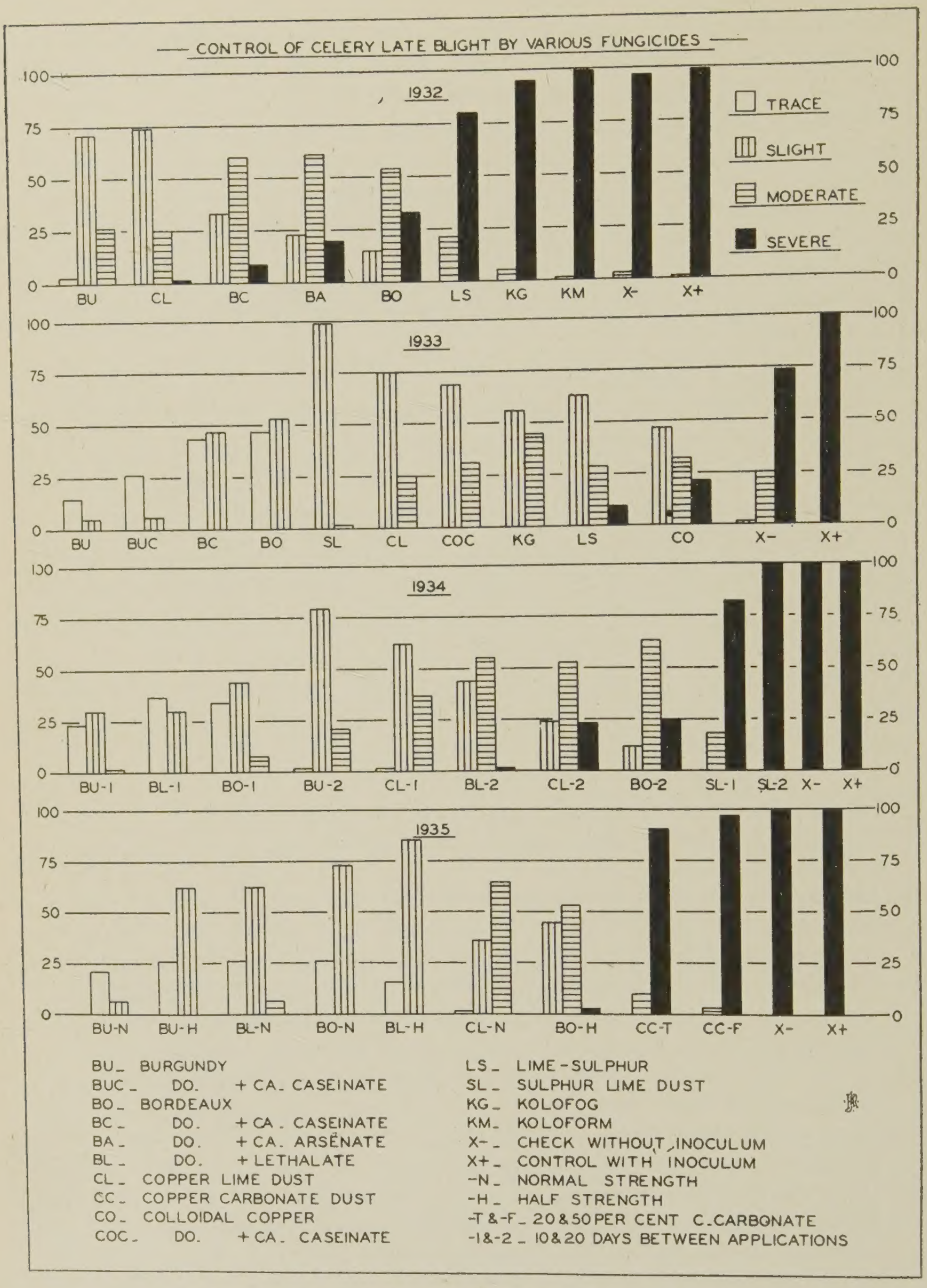
CONTROL OF CELERY LATE BLIGHT BY VARIOUS FUNGICIDES
1932
TRACE
SLIGHT
MODERATE
SEVERE
BU CL BC BA BO LS KG KM X- X+
1933
BU BUC BC BO SL CL COC KG LS CO X- X+
1934
BU-1 BL-1 BO-1 BU-2 CL-1 BL-2 CL-2 BO-2 SL-1 SL-2 X- X+
1935
BU-N BU-H BL-N BO-N BL-H CL-N BO-H CC-T CC-F X- X+
0 25 50 75 100
BU_ BURGUNDY
BUC_ DO. + CA_ CASEINATE
BO_ BORDEAUX
BC_ DO. + CA_ CASEINATE
BA_ DO. + CA_ ARSENATE
BL_ DO. + LETHALATE
CL_ COPPER LIME DUST
CC_ COPPER CARBONATE DUST
CO_ COLLOIDAL COPPER
COC_ DO. + CA_ CASEINATE
LS_ LIME-SULPHUR
SL_ SULPHUR LIME DUST
KG_ KOLOFOG
KM_ KOLOFORM
X-_ CHECK WITHOUT INOCULUM
X+_ CONTROL WITH INOCULUM
-N_ NORMAL STRENGTH
-H_ HALF STRENGTH
-T & -F_ 20 & 50 PER CENT C_CARBONATE
-1 & -2_ 10 & 20 DAYS BETWEEN APPLICATIONS

FIGURE 3.—The percentage of plants infected and the severity of late blight in plots of celery treated with various fungicides.

## RESULTS

As previously mentioned, significant results were not obtained in 1931. Frequent precipitation made conditions favourable for the development of the disease in 1932 but in 1933, due to drought during the growing season, there was considerably less blight in any of the plots. In 1934 and

1935 the disease was again prevalent, since natural precipitation was supplemented by overhead irrigation.

The quantity and severity of the disease in the plots showed a progressive increase with each of the recordings made during the summer, but the relative effectiveness of each fungicide remained the same. Only the final observations are considered here, because for our purposes it is the amount of disease present on the plants at harvest time that is of real commercial significance.

The accompanying graph (Figure 3) shows the percentage of plants infected and the severity of the disease in each of the plots, the unrecorded balance being disease-free. A clearer interpretation of the severity of the disease will be obtained by considering that all the plants having a slight infection, or less, are marketable as complete heads, those moderately diseased are still marketable as hearts, while severely diseased specimens are a total loss.

In 1932, Burgundy mixture and copper-lime dust gave the best results with 75% of the crop marketable as heads, and the remainder as hearts; but the former was slightly superior. Bordeaux plus calcium caseinate, Bordeaux plus calcium arsenate, and Bordeaux, were decreasingly effective in the order mentioned. Where sulphur fungicides were applied, from 80 to 99% of the plants were a total loss.

Although the entire series of plots were less severely diseased in 1933, nevertheless the various fungicides exhibited the same relative efficiency as in other years. Burgundy and Bordeaux mixtures, with and without calcium caseinate, all produced 100% marketable plants, but Burgundy mixture proved most effective. Sulphur-lime dust, copper-lime dust, colloidal copper plus calcium caseinate, and Kolofog, were decreasingly effective in the order given. Lime-sulphur, and colloidal copper, were the least effective, producing 56% and 47% marketable celery, respectively.

In 1934, Burgundy again gave the best control of the disease, with Bordeaux plus lethalate second, and Bordeaux third, all producing a crop of which over 90% was marketable. Copper-lime dust was next in order of merit, and sulphur-lime dust the least effective of the treatments. The control effected by applications at 20-day intervals was much inferior to that obtained for the shorter period, but the relative effectiveness of the various materials was the same.

Conditions during the summer of 1935 were favourable for the development of blight, and many growers who previously had little disease in their crops, reported severe losses. In the experiments conducted at the laboratory, the results corroborated those obtained in 1934, but the copper-lime dust did not give quite as good control as in previous years. Burgundy mixture was again the most efficient fungicide, and even when used half strength gave better protection than any other compound used. Bordeaux plus lethalate, and normal strength Bordeaux, also gave good commercial control. Copper carbonate-lime dust proved ineffective as a means of controlling the disease.

The inferior results obtained with the sprays in the first year, as compared with those of the three subsequent ones, were most probably due to the kind of sprayer used. In 1932, a small low-pressure machine

was the only equipment available, but during the following seasons, much better coverage was effected by the use of a more powerful sprayer which drove the fungicides well down into the hearts of the plants. Field observations have already demonstrated that adequate pressure during spraying operations cannot be too greatly stressed.

The results obtained from these tests have shown that even during seasons when weather conditions are favourable for the development of blight, the disease can be controlled satisfactorily by the thorough and frequent application of a suitable fungicide. Under conditions prevailing in the Niagara peninsula, the copper sprays, Burgundy and Bordeaux mixture, controlled the disease better than copper-lime dust. Local cultural conditions, and the availability of an adequate water supply, will determine whether dust or spray can be most satisfactorily applied. The addition of a spreader or sticker to Bordeaux mixture increased its effectiveness. The sulphur fungicides employed, and copper carbonate-lime dust, have very little fungicidal value as a control for late blight.

## CONTROL RECOMMENDATIONS

For the adequate control of celery blights, the following recommendations should be followed:

1. Use a good copper fungicide, either Burgundy mixture 4-5-40, or Bordeaux mixture 4-4-40 as a spray, or monohydrated copper sulphate plus lime 20-80 as a dust.

2. Start spraying early, giving the seedlings several applications before they are set into the field.

3. Apply fungicides thoroughly, and at sufficiently short intervals to keep the new growth covered.

4. Apply the fungicide before rainy periods whenever possible.

5. Do not wait until the disease appears before commencing regular spraying.

6. If dusting is being practised, make the applications as early as possible in the day, when the dew is still on the plants.

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# A GROWTH FACTOR INFLUENCING THE DEVELOPMENT OF *OPHIOBOLUS GRAMINIS* SACC.[1]

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[Received for publication October 26, 1935]

## INTRODUCTION

*Ophiobolus graminis* is one of a group of fungi which grow very poorly on a number of ordinary synthetic media, while growing excellently on some nutrient media containing plant or animal extracts. Broadfoot (*1*) wishing to find a medium on which *O. graminis* would grow well, tried a number of media, including Dox's inorganic salt; Brown's synthetic; Czapek's; Harvey's glucose-glycerol; Molisch's salt peptone; peptone sucrose; Waksman's sucrose peptone; Bezancon's glycerol peptone; Zike's magnesium sucrose asparagin; Woltze's sucrose ammonium sulphate; Heinemann's asparagin; Bokorny's ethyl alcohol ammonium sulphate and potato dextrose. It grew best on Molisch's salt peptone agar and on potato dextrose agar. The author has found that remarkably good growth occurs on carrot dextrose agar. Ezekiel, Taubenhaus and Fudge (*5*) similarly found that carrot extract is particularly suitable for growth of *Phymatotrichum omnivorum*.

Farries and Bell (*6*) were unable to grow *Nematospora gossypii* on a synthetic solution, and believing that the nitrogen source was perhaps unsuitable, tried numerous nitrogenous materials. Many of them resulted in excellent growth, but it was remarked that the only materials promoting growth were natural proteins. Various nitrogen salts and pure amino-acids failed completely. They therefore set out to try to discover the reason for this.

Peptone gave excellent growth, and Farries and Bell purified the protein of peptone by precipitating with alcohol. The pure protein failed to give growth, but when mixed with the filtrate growth resulted. At this point they discarded peptone and worked with egg albumen, which gave similar results. Realizing that some impurity occurred with the proteins which was essential for growth, they referred to a specific substance which they called a "growth factor". This substance they were able to concentrate by a series of fractionations. It remained in the alcohol filtrate after precipitating the proteins, and was insoluble in ether and absolute alcohol. It was not precipitated with phosphotungstic acids, and was therefore presumably not a base. It was thrown down, along with sugars, on addition of lead acetate.

The further biochemical analysis was continued by Buston and his co-workers. Using lentils as the source of growth factor, they precipitated the proteins with alcohol, granulated the filtrate with acetone, and precipitated with baryta. It was found that baryta divided the growth factor into two portions, both necessary for *N. gossypii*. The active part of the precipitate Buston and Pramanik (*2*) proved to be inositol. It was pointed out (*3*) that the active portion of the filtrate ("factor II") does not possess the properties of "bios".

[1]Portion of a thesis accepted for the degree of Doctor of Philosophy at the University of London.

By treating baryta filtrate with phosphotungstic acid Buston and Kasinathan (*4*) proved that factor II is present in two forms, that in the precipitated portion being (probably loosely) combined with a base, while the filtrate part is in a free form possessing properties suggestive of an acid.

## EXPERIMENTAL

### A. *General experimental method*

In the experiments described here *O. graminis* was cultured on media of pH 6.0 to 6.5 at 25°C. These conditions have been found favorable and are easily obtained. In the work dealing with *Nematospora gossypii*, cited above, the fungus was grown on agar and on completion of the experiment it was scraped off the surface with a spatula, dried, and weighed. The applicability of this method depended upon the fact that with that fungus the amount of mycelium imbedded within the agar (and therefore not recovered) was negligible. In the case of *O. graminis*, a large proportion of the mycelium was imbedded, so that that method could not be used. The cultures were therefore grown on liquid substrates. For this purpose 20 c.c. of the solution were placed in medicine bottles so that when these were laid on their sides the layer of liquid was quite thin and therefore well aerated. The basal medium employed had the following composition:

| | |
|---|---|
| Glucose | 20 gms. |
| Nitrogen Source | —See below |
| $KH_2PO_4$ | 5 gms. |
| $MgSO_4$ | 2.5 gms. |
| $FeCl_3$ | a trace |
| Water | 1 litre |

In the experiments with casein extract the nitrogen content of the solution was brought to 0.15% with sodium nitrate after allowing for the total nitrogen contents of any extracts added, as determined by the micro-kjeldahl method (*8*). In experiments with carrot extract 0.15% nitrogen was added as sodium nitrate and the nitrogen in the extract was disregarded.

The pH's of the solutions were adjusted with normal sodium hydroxide and hydrochloric acid so that they were approximately equal after autoclaving, varying in different experiments from 6.0 to 6.7.

The solutions were inoculated with small pieces of potato dextrose agar supporting a week-old culture of *O. graminis* and were incubated at 25°C. for 10 or 14 days. The mycelium was then removed, washed, dried and weighed, all the replicates of one treatment being bulked. The resemblance between replicate flasks was always very close. The pH of each solution was determined after completion of the experiment.

### B. *Reaction to the accessory growth factor of casein*

An extract of commercial casein was made by steaming it for one hour in distilled water and filtering. The insoluble portion was discarded. The filtrate was then freed from proteins by three successive precipitations with three times its bulk of 95% alcohol. The proteins were purified by two further precipitations (Material A). The filtrate was extracted four times with ether, twice in acid and twice in alkaline solution and the ether-soluble fractions (containing fats and simple amino acids) discarded. The

ether-insoluble fraction was evaporated to dryness and extracted with 95% alcohol, giving soluble (B) and insoluble (C) fractions.

In this experiment the sources of nitrogen used were:

(1) Crude casein, unprecipitated.
(2) Precipitated protein (Fraction A).
(3) Sodium nitrate.

The extracts used were:

(1) None.
(2) Fraction B.
(3) Fraction C.
(4) Fractions B+C.

While the fractions B and C used were proportionately equal, they were not however equivalent to the amount present in crude casein (i.e., solution 1). In all cases the nitrogen content was 0.15%, and the pH was adjusted to about 6.2. Table 1 shows the yield of dried mycelium.

TABLE 1—EFFECT OF CASEIN FRACTIONS ON GROWTH OF *O. graminis*

| Nitrogen source | Fraction | Final pH | Dry wt. of mycelium mgms. |
|---|---|---|---|
| Crude casein | None | 6.0 | 190 |
| | B | 6.0 | 190 |
| | C | 6.0 | 190 |
| | B+C | 6.0 | 210 |
| Precipitated protein (Fraction A) | None | 6.0 | 3 |
| | B | 6.0 | 35 |
| | C | 6.0 | 70 |
| | B+C | 6.0 | 110 |
| Sodium nitrate | None | 6.4 | 3 |
| | B | 6.4 | 55 |
| | C | 6.4 | 25 |
| | B+C | 6.4 | 70 |

The yields of mycelium show without any doubt that in casein there is some substance essential for the growth of *O. graminis* which is not present in either the synthetic basal solution or purified protein from the casein. This substance is to some extent soluble in 95% alcohol, which was also the case with the extracts of egg albumen which Farries and Bell found to give good growth of *Nematospora gossypii*. Except in the presence of crude casein, the sum of yields with fractions B and C approximately equals the yield when these two fractions are recombined, suggesting that they are supplementary rather than complementary.

Other experiments, conducted with the alcohol insoluble fraction only, all gave similar results with *O. graminis*, and also with *Nematospora gossypii* and *Melanospora destruens*. One fungus, however, *Penicillium italicum*, gave little response to the growth factor, excellent growth resulting in its absence. In other experiments various nitrogen sources were used to replace the sodium nitrate in the synthetic medium, including potassium nitrate, ammonium sulphate, ammonium nitrate, asparagin and urea, but in all cases growth of *O. graminis* was negligible in the absence of the extract. Replacement of glucose by sucrose or fructose also failed to promote growth.

C. *Reaction to extracts from carrots*

The soluble portion obtained when carrots were steamed with distilled water in an autoclave for one hour was filtered, and the filtrate was freed from proteins by two successive precipitations with alcohol. The filtrate was reduced in volume and was found to be acid in reaction. It was shaken three times with ether for removal of fats, the insoluble fraction being retained, reduced in volume to a thick syrup, shaken with acetone and redissolved in water. Half of this was preserved and called "fraction 1".

The other half was saturated with barium hydroxide and precipitated with three times its volume of alcohol. This is a standard method of precipitating inositol. Both precipitate and filtrate were freed from barium with sulphuric acid, and are named respectively "fraction 2" and "fraction 3".

All three fractions were made up to the same volume and are therefore the extracts from corresponding quantities of material.

In the first experiment with this material the extracts were used in the quantities 7.5, 15 and 30 c.c. per litre of solution, corresponding to ½, 1 and 2 times the equivalent of 200 grams of carrots per litre. In addition, pure inositol, which Buston and Pramanik have found to be one of the two complementary growth factors, was used in several quantities. The extracts were not analysed for nitrogen. Instead, the various extracts were added to the basal solution in which (a) nitrogen or (b) glucose was lacking. It was considered that the available nitrogen and sugar contents of the extracts could then be allowed for in the calculations. The pH was adjusted to 6.7. The growth of the fungus after 14 days is shown in Table 2.

TABLE 2.—EFFECT OF CARROT FRACTIONS ON GROWTH OF *O. graminis*

| Culture soln. | Substances added per litre | Final pH | Dry wt. of mycelium mgms. |
|---|---|---|---|
| Basal+$NaNO_3$ | None | 6.7 | 2 |
| Basal+$NaNO_3$ | Inositol, 0.05 gm. | 6.7 | 2 |
| Basal+$NaNO_3$ | Inositol, 0.10 gm. | 6.7 | 3 |
| Basal+$NaNO_3$ | Inositol, 0.20 gm. | 6.7 | 2 |
| Basal+$NaNO_3$ | Inositol, 0.40 gm. | 6.7 | 3 |
| Basal+$NaNO_3$ | Baryta ppt., 7.5 c.c. | 6.5 | 86 |
| Basal+$NaNO_3$ | Baryta ppt., 15.0 c.c. | 6.5 | 118 |
| Basal+$NaNO_3$ | Baryta ppt., 30.0 c.c. | 6.3 | 168 |
| Basal+$NaNO_3$ | Baryta filr. 7.5 c.c. | 8.0 | 649 |
| Basal+$NaNO_3$ | Baryta filr. 15.0 c.c. | 8.0 | 687 |
| Basal+$NaNO_3$ | Baryta filr. 30.0 c.c. | 7.6 | 643 |
| Basal+$NaNO_3$ | Unpreciptd. fraction, 7.5 c.c. | 8.0 | 786 |
| Basal+$NaNO_3$ | Unpreciptd. fraction, 15.0 c.c. | 8.3 | 910 |
| Basal+$NaNO_3$ | Unpreciptd. fraction, 30.0 c.c. | 8.0 | 985 |
| Basal only | Baryta ppt., 15.0 c.c. | 6.6 | 99 |
| Basal only | Baryta filtr. 15.0 c.c. | 6.7 | 164 |
| Basal only | Unpreciptd. 15.0 c.c. | 6.9 | 420 |
| Basal+$NaNO_3$−glucose | Baryta ppt., 15.0 c.c. | 6.5 | 113 |
| Basal+$NaNO_3$−glucose | Baryta filtr., 15.0 c.c. | 6.8 | 65 |
| Basal+$NaNO_3$−glucose | Unpreciptd. 15.0 c.c. | 8.0 | 210 |

An examination of Table 2 reveals the following facts.

(1) In the absence of extracts growth was negligible.

(2) Inositol failed to stimulate growth.

(3) Growth in presence of "baryta precipitate" was proportional to the amount present. "Baryta filtrate" gave much greater stimulation, and growth was not proportional to the amount present.

(4) Growth in presence of the unprecipitated fraction was slightly greater than the sum of the growths with the corresponding quantities of "baryta precipitate" and "baryta filtrate".

(5) With all three fractions some growth occurred in the absence of nitrogen or glucose. The "baryta filtrate" proved to be low in available sugars, and the precipitate was low in available nitrogen. Sodium nitrate is seen to be an excellent source of nitrogen for the fungus.

The moderately good growth with the baryta precipitate can be explained if we assume that separation was not complete. It is worth noting that where growth was substantial the pH had changed to about 8.0, which in preliminary experiments was found to be approaching the upper limit for the rapid growth of the fungus. It is therefore probable that none of the nutrients were limiting factors even where heaviest growth occurs, a fact which justifies the omission of accurate adjustment of the nitrogen content.

Comparatively poor growth with baryta precipitate alone may be assumed to be due mainly to lack of sufficient growth factor. It cannot be due to presence of any toxic substance since growth was directly proportional to the quantity of extract present. The failure of the sum of growths with filtrate and precipitate to equal that of the unseparated fraction may be attributed to loss of a small portion of the effective extract in removing the barium sulphate.

A portion of the baryta precipitate fraction was reprecipitated with barium hydroxide and alcohol in an attempt to free it further of the active substance, and the baryta filtrate fraction was also reprecipitated. After removing the barium these fractions were brought back to their original volumes.

The purified precipitate and filtrate were compared with the unprecipitated fraction; the two were recombined; and inositol was added to the purified baryta filtrate. Basal solution with $NaNO_3$ was used and the pH was adjusted to 6.5. Results are given in Table 3.

TABLE 3.—EFFECT OF REPRECIPITATION WITH BARYTA ON "BARYTA FILTRATE" AND "BARYTA PRECIPITATE"—BASAL SOLUTION AND SODIUM NITRATE

| Fraction | Final pH | Dry wt. of mycelium, mgms. |
|---|---|---|
| Purified baryta ppt. | 6.2 | 52 |
| Purified baryta filtrate | 6.7 | 294 |
| Purified baryta ppt. + purified baryta filtrate | 6.9 | 510 |
| Inositol 0.5 grams per litre + purified baryta filtrate | 6.7 | 284 |
| Unprecipitated fraction | 8.0 | 1105 |
| None | 6.4 | 3 |

By the second precipitation much of the growth factor appears to have been lost, or else some toxic substance was left which inhibited to some extent the growth of the fungus. It is noticeable here that inositol

failed to enhance the effect of purified baryta filtrate. On the other hand, purified baryta precipitate, which caused little growth of itself, gave a rather substantial increase to the effectiveness of the purified baryta filtrate. Without further experiments it would be unwise to speculate on the significance of this result.

An attempt was made to remove some of the nitrogen from the baryta filtrate. Bases readily form insoluble phosphotungstates. A portion of the filtrate was treated with 20% phosphotungstic acid in 5% sulphuric acid, and both filtrate and precipitate were decomposed with barium hydroxide. As quickly as possible the barium was removed with sulphuric acid. Using basal solution of pH 6.4 lacking nitrogen the phosphotungstic acid filtrate and precipitate and the two combined were compared with baryta filtrate (Table 4).

TABLE 4.—REMOVAL OF BASES BY PHOSPHOTUNGSTIC ACID—BASAL SOLUTION LACKING NITROGEN

| Fraction | Final pH | Dry wt. of mycelium mgms. |
|---|---|---|
| Original baryta filtrate | 6.7 | 154 |
| Phosphotungstic acid filtrate | 6.6 | 99 |
| Phosphotungstic acid ppt. | 6.6 | 52 |
| Phosphotungstic acid filtrate+ppt. | 6.7 | 143 |
| None | 6.5 | 12 |

Precipitation with phosphotungstic acid removed only a small proportion of the nitrogen, which was readily replaced by recombining the filtrate and the precipitate with the result that growth was equal to the original baryta filtrate.

D. *Growth in the Presence of Bacteria*

In an early experiment a bottle of *O. graminis* in basal solution was found contaminated with bacteria. In this bottle *O. graminis* grew well, but in the similarly treated bottles which remained uncontaminated growth was negligible.

A culture of these unidentified bacteria was preserved and repeatedly sub-cultured. After many months it was found that *O. graminis* was still present with the bacteria, and after two or three more sub-cultures were made the bacteria were obtained free from *O. graminis* by suspending in water and streaking on agar. Bottles of basal solution were divided into 2 lots, one inoculated with the bacteria and the other not. Substantial growth of the bacteria occurred, and after 9 days they were killed by autoclaving. These bottles, as well as those in which the bacteria had not grown, were inoculated with *O. graminis* and incubated at 25°C. for 21 days.

The mycelium was dried and weighed, the yield in bottles which had grown bacteria being 150 mgms., whereas in those without bacteria it was only 5 mgms. Evidently the bacteria produced the growth factor for *O. graminis* or dispensed in some way with its necessity.

## DISCUSSION

Nutritional aspects of *O. graminis* have been to some extent neglected owing to the interesting phenomenon of antagonism of other organisms towards it. Sanford and Broadfoot (*9*), who have done much work on the problem of antagonism, seem of the opinion that since *O. graminis* will

grow well in sterilized soil it should be able to grow well also in unsterilized soil, and conclude that the reason why it does not do so is that other organisms are antagonistic towards it. In a measure this is doubtless true, since many fungi and bacteria do inhibit under certain conditions the growth of this fungus. On the other hand it has by no means been proved that ordinary unsterilized soil is of itself a good medium for *O. graminis* even in the absence of other organisms or toxic substances produced by them. It has been stated by Buston and his workers that some organisms readily decompose the growth factor of *Nematospora gossypii*, and doubtless such organisms occur in the soil. Thus it may well be that the quantity of growth factor in the soil in the form of decaying plant material remains at a low level. Furthermore, since this growth factor is largely released by heating, sterilization of soil probably promotes abnormally good growth of *O. graminis*. In any case its growth even in most sterilized soils is very meagre unless some form of plant extract is added. It is as reasonable to suppose that *O. graminis* fails to grow in unsterilized soil, at least in some cases, through lack of growth factor, or through competition for growth factor by other organisms, as it is to regard the control exercised as being entirely due to production of some inhibitory substance by these organisms. The writer suggests that results of his previous experiments (*7*) showing the inability of *O. graminis* to spread appreciably in unplanted soil are due to both antagonism of other organisms and lack of growth factor.

Although Sanford and Broadfoot (*1*, *9*) find some organisms which stimulate the attack of wheat by *O. graminis*, they nevertheless divide soil micro-organisms into only two classes, namely

(*a*) Those which are antagonistic towards *O. graminis*.

(*b*) Those which are compatible with *O. graminis*.

The third group receives no place in the classification. It is of interest to note in this connection that Van Luijk (*10*) has recently discovered certain saprophytes which intensify the attack of grasses by species of *Pythium*.

The experiments described here and the fractionations which were conducted may appear somewhat inadequate from a chemical aspect; further work may well be left to the biochemist. What has been demonstrated is the necessity of a growth promoting factor for *Ophiobolus graminis*, and consequently the importance of studying the nutritional aspect when considering questions of antagonism and growth of the fungus in soil.

## SUMMARY

(1) *O. graminis* is unable to grow in some common synthetic solutions without the addition of certain plant or animal extracts.

(*2*) Casein was extracted with water, the proteins precipitated with alcohol, and the ether-soluble fraction removed. The extract still remained active in promoting growth.

(3) Carrots were extracted with water, the proteins precipitated, ether-and acetone-soluble fractions removed, and much of the sugar precipitated with barium hydroxide. The extract still remained very active in promoting growth. This preparation provided very little available sugar and a small quantity of nitrogen.

13640—2½

(4) Further experiments would be necessary to determine whether the growth factor concerned is identical with the second growth factor found by Buston *et al* to be necessary for *Nematospora gossypii*.

(5) A culture of bacteria was found which replaces the growth factor in synthetic solutions.

(6) The growth factors probably play an important part in maintaining the balance of micro-organisms in the soil.

ACKNOWLEDGMENT

The author wishes to thank Prof. W. Brown for providing facilities for conducting this work at the Imperial College of Science and Technology and for advice in preparing the manuscript.

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## Résumé

**Un facteur de végétation influençant le développement de *Ophiobolus graminis* Sacc. par G. W. Padwick, Station de recherches agricoles de Jealott's Hill, Bracknell, Berks., Angleterre.**

*O. graminis* ne peut se développer dans certaines solutions synthétiques communes sans l'addition de certains extraits végétaux ou animaux. Après extraction de la caséine avec de l'eau, précipitation des protéines au moyen d'alcool et enlèvement de la fraction soluble dans l'éther, l'extrait exerce encore une action stimulante sur la végétation. Après extraction de la protéine des carottes avec de l'eau, précipitation des protéines et enlèvement des fractions solubles dans l'éther et l'acétone, et précipitation d'une bonne partie du sucre au moyen d'hydroxide de barium, l'extrait est encore très actif. Cette préparation n'a fourni qu'une très petite quantité de sucre et une petite quantité d'azote. Il semble que de nouvelles expériences seraient nécessaires pour voir si le facteur de végétation à l'étude est identique au facteur de deuxième végétation, que Buston et d'autres ont trouvé nécessaire pour *Nemastospora gossypii*. On a trouvé une culture bactérienne qui remplace le facteur de végétation dans les solutions synthétiques. Il est probable que les facteurs de végétation jouent un rôle important en maintenant l'équilibre des micro-organismes dans la terre.

# METHODS OF CARING FOR FARM SEPARATORS AND THEIR INFLUENCE UPON THE QUALITY OF CREAM[1]

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[Received for publication March 6, 1936]

Contamination from improperly cared for separators is undoubtedly a major factor in lowering the grade of cream for butter-making. Where only a small mount of milk is separated, the work involved in washing the separator after each milking often appears to the farmer to be greater than is economically justifiable. In a survey conducted in Idaho (*1*) only 7 out of 89 separators were always washed after each time they were used, and a similar proportion would doubtless be found in many other districts. While recognizing that best results can only be obtained where the separator is thoroughly washed and sterilized after each separation, it seemed worth while to explore the possibilities in the way of simpler methods of caring for farm separators in the hope that the present degree of contamination might thus be appreciably reduced.

Where the separator bowl is not taken apart and washed after the evening milking, the bacteria present in the milk residue and separator slime find abundant food and moisture for growth. If favourable temperatures are encountered, multiplication proceeds rapidly and enormous numbers of bacteria will be present in the bowl next morning. This growth could be prevented by holding the bowl at a sufficiently low temperature overnight, but unfortunately refrigerated storage is rarely available for the smaller cream producer. Among the more practicable measures for reducing contamination which suggest themselves are: (1) leaving the bowl filled overnight with some bacteriostatic solution, (2) flushing with cold water after the evening and before the morning separation, and (3) flushing with hypochlorite solution. Studies were therefore conducted to determine the relative efficiency of these various measures, taking care to simulate actual farm conditions as closely as possible in the handling of the separator, storage of cream, etc.

## EXPERIMENTAL

### Part I

A new hand separator[3] of 500 lbs. capacity was used to separate sixteen gallons of freshly drawn milk from the Central Experimental Farm herd at each milking. After each morning's separation, the machine was taken apart, all parts thoroughly washed, steamed and allowed to dry. Treatments after the evening's separation and before the morning's are described separately in each series. Samples of whole milk and cream were taken for bacterial analysis, and plated out on nutrient agar (Difco) plus 0.5 per cent skim milk. Evening's samples were incubated for 65 hours and

[1] Contribution No. 36 (Journal Series) from the Division of Bacteriology, Dominion Experimental Farms, Ottawa.

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[3] Supplied through the courtesy of the De Laval Co. Ltd., Peterborough, Ontario.

morning's for 48 hours, at 32° C. (*15*). After plating, cream samples were held at approximately 15.5° C. and titrated for acidity each morning, using 9 cc. diluted with a similar volume of distilled water as recommended by Sommer (*17*). To enable the estimation of the relative keeping quality, curves were plotted for each sample from which the number of hours required to show 0.2% increase in acidity were read off. This represents approximately the increase which would lower the grade of clean flavoured cream from Special to No. 1, where the dividing line is set at 0.3% acidity.

## Series I

*A. Metso Solution.* Metso (sodium metasilicate) has been shown (*9*) to be quite effective in keeping down growth of bacteria in milking machine rubber parts, while the manufacturers, supported by statements in a technical paper (*2*) claim a much lower degree of corrosiveness for solutions of this product than for other alkaline detergents. Consequently an attempt was made to determine the bacteriostatic and detergent value of this material in the treatment of separators. After a few preliminary studies a series of tests was conducted in which 2 quarts of 0.5% Metso solution at approximately 60° C. were poured into the supply can immediately after the evening's milk had passed through the machine. The bowl remained filled with this solution until morning, the temperature of the room fluctuating between 13 and 18° C. during the night. The supply can, spouts, etc. were rinsed off in cold water. Next morning the contents of the bowl were drained out, the separator re-assembled, and when the proper speed had been attained two quarts of cold tap water were run through ahead of the milk. Data obtained are given in Table 1, under Series I, Section A. Unfortunately, corrosion of the bowl and discs was so marked within a few days that the treatment had to be discontinued.

*B. Cold Water.* These tests were conducted in order to determine the value of flushing with two quarts of water at around 8° C. combined with holding the bowl overnight in a room where the temperature varied between 10 and 20° C. In all other respects the methods employed were identical with those described in Section A. The data are presented under Section B, Series I in the table.

## Series II

*A. Cold Water.* In this series several changes were made. In order to effect a more complete removal of the residue, the supply can and bowl were flushed out with two gallons[1] of cold water (4° C.) after the evening's and before the morning's separation. Furthermore, in order to study the influence of a higher overnight temperature, the bowl was drained and held in an inverted position for 14 hours at approximately 25° C. Plating and titrations were conducted as in Series I. Data are shown under Series II A in Table 1.

*B. Hypochlorite.* In these tests two gallons of 50 p.p.m. solution of NaOCl[2] of low alkalinity were flushed through just before the morning separation. No other change was made. Data appear under Series II B in Table 1.

[1] Imperial measure (1 gallon = 1.20094 U.S. gallons).

[2] A stock solution prepared from B-K powder and containing 10,000 p.p.m. Av. Cl. was diluted 1/200 with cold water.

TABLE 1.—EFFECTIVENESS OF VARIOUS SEPARATOR TREATMENTS AS JUDGED BY BACTERIAL COUNT AND KEEPING TIME (RATE OF ACID DEVELOPMENT) OF CREAM AT 15.5° C.

| | No. of trials | P.M. samples (washed separator) | | | | A.M. samples (unwashed separator) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Milk | Cream | | | Milk | Cream | | |
| | | *Plate count | *Plate count | Fat % | Keeping time (hrs.) | *Plate count | *Plate count | Fat % | Keeping time (hrs.) |
| *Series I* (Nov.–Dec. 1934) | | | | | | | | | |
| A. hot Metso sol'n p.m. cold water a.m. | 5 | 5,915 | 5,915 | 33.8 | 77.6 | 10,716 | 7,870 | 34.0 | 72.8 |
| B. cold water p.m. cold water a.m. | 4 | 5,041 | 5,159 | 33.5 | 83.5 | 12,955 | 17,985 | 29.0 | 69.3 |
| *Series II* (Jan. 1935) | | | | | | | | | |
| A. cold water p.m. cold water a.m. | 3 | 1,935 | 2,308 | 35.0 | 57.7 | 4,814 | 101,800 | 34.3 | 44.6 |
| B. cold water p.m. NaOCl a.m. | 3 | 2,493 | 1,080 | 39.3 | 79.0 | 5,209 | 5,889 | 38.3 | 70.5 |

* = Logarithmic average.

## DISCUSSION

It would seem that the temperature at which the bowl is held overnight is the chief factor influencing the bacterial contamination of the cream from an unwashed separator. Where the bowl was held overnight at 10 to 20° C. there was no marked increase in count even when the bowl was merely flushed with 2 quarts of cold water after the evening and before the morning milking. When, however, the bowl was held overnight at 25° C. marked contamination followed, even though four times the quantity of water was flushed through the separator. The substitution of sodium hypochlorite for cold water in flushing the bowl before the morning separation greatly reduced the bacterial contamination. No reason has been found for the abnormally rapid increase in acidity in the evening's cream, Series II A.

Metso solution allowed to remain in the bowl overnight exhibited satisfactory bacteriostatic activity. Platings from the material drained from the bowl next morning indicated a count even lower than that of the milk separated the previous evening. Nevertheless the marked corrosive action of this solution upon the discs, etc. makes it totally unsuitable for this purpose. Subsequent tests with tinned steel strips half immersed in 0.5 per cent solutions of various alkaline detergents for 48 hours at 28° C. (Table 2) indicated that the loss of weight with Metso was intermediate between the values for sodium hydroxide and trisodium phosphate, in agreement with the relative alkalinity of these products.

From the data summarized in Table 1, it will be observed that the acidity increased more rapidly in the morning's cream than in the evening's cream of comparable bacterial content. Several possible explanations suggest

themselves. In the first place, the organisms entering from the residue in the unwashed bowl at the morning's separation may be less inhibited by the action of the lactenin in the freshly separated cream, in the same manner as the mastitis streptococci (*13*). Again there may be a difference

TABLE 2.—CORROSION OF TINNED STEEL BY ALKALINE DETERGENT SOLUTIONS

A. Strips half immersed in 0.5% solutions at 28° C.

| | NaOH | | Metso | | $Na_3PO_4,12H_2O$ | | $Na_2CO_3$ | |
|---|---|---|---|---|---|---|---|---|
| Wt. at start | 9.1631 | 8.8834 | 9.1891 | 9.3509 | 9.0005 | 9.3425 | 9.3861 | 9.3314 |
| Wt. after 48 hrs. | 9.0923 | 8.8057 | 9.1365 | 9.3028 | 8.9687 | 9.3089 | 9.3700 | 9.3118 |
| Loss in weight (gms.) | 0.0708 | 0.0774 | 0.0526 | 0.0481 | 0.0318 | 0.0336 | 0.0161 | 0.0196 |

B. Strips half immersed in 0.2% Solution at 28° C.

| | | | Metso | | | | | |
|---|---|---|---|---|---|---|---|---|
| Wt. at start | | | 9.2411 | 9.4225 | | | | |
| Wt. after 48 hrs. | | | 9.2035 | 9.3820 | | | | |
| Loss in weight (gms.) | | | 0.0376 | 0.0405 | | | | |

in the flora of the evening's and morning's milk, although results obtained by fishing colonies to litmus milk have been inconclusive. Finally there may be some inherent difference between evening's and morning's milk which affects the growth of the milk-souring organisms. Reported data on this point are conflicting. Barkworth (*4*) in the South of England reported that on an average, morning's milk kept sweet nine hours longer than evening's milk of equivalent bacteria content. On the contrary, Thomas (*18*) reported that in a study of 2,225 samples in Wales, evening's milk kept sweet from three to twelve hours longer than morning's. This difference was attributed by Thomas to the higher temperature of the latter as received for analysis. In an endeavour to throw some light upon this matter and to gather further data concerning the effectiveness of these simpler methods of caring for separators, the studies reported in Part II were undertaken some months later.

## EXPERIMENTAL

### Part II

The first can of milk filled at each milking was taken for separation. Since the cows were milked in regular order, this represented approximately the milk from the same four cows at each milking. Treatment of the separator after the morning's milking was the same as described in Part I, while treatments subsequent to the evening's separation are described separately in each series. Analytical procedures for the cream were identical with those previously described, except that all plates were counted after 48 hours incubation at 32° C. The rate of acid development was followed in both milk and cream. For acidity determinations of milk a nine cc. sample, undiluted, was titrated with N/10 NaOH. Tubes of

TABLE 3.—DATA FROM SERIES A. SEPARATOR WASHED AND STEAMED AFTER EACH MILKING

| Date | Milk | | | | | | | | | | | | Cream | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A.M. Samples | | | | | | P.M. Samples | | | | | | A.M. Samples | | | | | | P.M. Samples | | | | | |
| | Keeping time (hrs.) | Fat % | Plate count | Coliform organisms in | | | Keeping time (hrs.) | Fat % | Plate count | Coliform organisms in | | | Keeping time (hrs.) | Fat % | Plate count | Coliform organisms in | | | Keeping time (hrs.) | Fat % | Plate count | Coliform organisms in | | |
| | | | | 1 cc. | 0.1 cc. | 0.01 cc. | | | | 1 cc. | 0.1 cc. | 0.01 cc. | | | | 1 cc. | 0.1 cc. | 0.01 cc. | | | | 1 cc. | 0.1 cc. | 0.01 cc. |
| | | | | % | % | % | | | | % | % | % | | | | % | % | % | | | | % | % | % |
| April 15 | | | | | | | 77 | ? | 2,500 | ? | ? | ? | | | | | | | 94 | 48 | 1,250 | ? | ? | ? |
| April 16 | 86 | 3.6 | 3,650 | ? | ? | ? | 80 | 4.0 | 1,400 | + | − | | 93 | 44 | 1,250 | ? | ? | ? | 84 | 44 | 450 | − | − | |
| April 17 | 85 | 3.4 | 6,700 | − | − | | 72 | 4.0 | 3,250 | + | + | − | 77 | 27 | 3,200 | − | − | | 66 | 38 | 1,450 | + | + | + |
| April 18 | 108 | 3.4 | 4,750 | − | − | − | 78 | 4.2 | 1,650 | − | − | | 74 | 30 | 2,550 | + | − | | 78 | 34 | 2,100 | − | − | |
| April 19 | 83 | 3.6 | 6,350 | − | − | | 83 | 4.4 | 4,150 | − | − | | 95 | 40 | 2,375 | − | − | | 105 | 32 | 1,400 | − | − | |
| April 20 | 89 | 3.0 | 8,100 | − | − | − | | | | | | | 69 | 34 | 4,050 | + | + | − | | | | | | |
| April 21 | | | | | | | 80 | 3.8 | 8,450 | + | − | − | | | | | | | 90 | 44 | 3,700 | + | − | − |
| April 22 | 90 | 3.6 | 20,000 | + | + | − | 77 | 4.0 | 4,225 | − | − | − | 85 | 36 | 5,150 | + | − | − | 76 | 44 | 1,500 | − | − | − |
| April 23 | 71 | 3.2 | 3,500 | − | − | − | | | | | | | 70 | 35 | 875 | − | − | − | | | | | | |
| Average | 87.4 | 3.37 | 6,351* | 16.7 | 16.7 | 0.0 | 78.1 | 4.07 | 3,121* | 50.0 | 16.7 | 0.0 | 80.4 | 35.1 | 2,400* | 50.0 | 16.7 | 0.0 | 84.7 | 40.6 | 1,445* | 33.3 | 16.7 | 16.7 |

* = Logarithmic average.

TABLE 4.—DATA FROM CREAM SAMPLES, SERIES B
(Bowl held overnight at 28° C.)

| Date | Separator washed and steamed (P.M. milking) | | | | | | Separator flushed with cold water after P.M. and before A.M. run | | | | | | Separator flushed with cold water after P.M. and with NaOCl before A.M. run | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Keeping time (hrs.) | Fat % | Plate count | Coliform organisms in | | | Keeping time (hrs.) | Fat % | Plate count | Coliform organisms in | | | Keeping time (hrs.) | Fat % | Plate count | Coliform organisms in | | |
| | | | | 1 cc. | 0.1 cc. | 0.01 cc. | | | | 1 cc. | 0.1 cc. | 0.01 cc. | | | | 1 cc. | 0.1 cc. | 0.01 cc. | 0.001 cc. |
| | | | | % | % | % | | | | % | % | % | | | | % | % | % | % |
| April 23 | 67 | 33 | 1,675 | — | — | — | | | | | | | | | | | | | |
| April 24 | 91 | 42 | 1,450 | — | — | — | | | | | | | | | | | | | |
| April 25 | 68 | 38 | 1,200 | — | — | — | 50 | 36 | 337,000 | + | + | — | 72 | 33 | 18,575 | + | + | + | — |
| April 26 | 88 | 40 | 700 | — | — | — | | | | | | | | | | | | | |
| April 27 | | | | | | | 69 | 35 | 127,000 | + | — | — | 70 | 36 | 15,700 | + | + | + | — |
| April 28 | 88 | 34 | 4,600 | — | — | — | | | | | | | | | | | | | |
| April 29 | 88 | 41 | 1,400 | — | — | — | | | | | | | | | | | | | |
| April 30 | 84 | 38 | 1,500 | — | — | — | 65 | 38 | 55,500 | + | + | — | 75 | 34 | 55,000 | + | + | — | — |
| May 1 | 87 | 33 | 2,100 | — | — | — | | | | | | | | | | | | | |
| May 2 | 85 | 35 | 3,300 | — | — | — | 64 | 30 | 52,000 | + | + | — | 75 | 35 | 16,050 | + | — | — | — |
| May 3 | 84 | 35 | 3,750 | — | — | — | | | | | | | | | | | | | |
| May 4 | 83 | 35 | 3,000 | — | — | — | 65 | 31 | 140,000 | + | + | — | 77 | 30 | 12,300 | — | — | — | — |
| May 5 | 79 | 36 | 950 | — | — | — | | | | | | | | | | | | | |
| May 6 | | | | | | | 53 | 32 | 57,000 | + | — | — | 73 | 33 | 56,000 | — | — | — | — |
| Average | 82.7 | 36.7 | 1,838* | 0.0 | 0.0 | 0.0 | 61 | 33.7 | 100,000* | 100 | 66.7 | 0.0 | 73.7 | 33.5 | 23,800* | 66.7 | 50.0 | 33.3 | 0.0 |

* = Logarithmic average.

brilliant green lactose bile broth (Difco) were inoculated to determine the presence of coliform organisms, since English workers (*3*) have affirmed that keeping quality is deleteriously affected by their presence.

## Series A

In the hope of determining whether or not there is any intrinsic difference in the rate of acid formation in evening's and morning's milk and cream, the separator was washed and steamed after each milking for seven consecutive days. The data obtained are presented in Table 3 When the differences in bacterial and fat content are considered, the advantage of 4·3 hours in favour of the evening's cream samples seems not unreasonable. The evening's milk samples on the other hand, show an average keeping time of 9.3 hours *less* than the morning's, despite a considerably lower bacterial count and presumably higher buffer capacity (as judged by the butterfat content). Coliform organisms were somewhat more abundant in the evening's milk samples, although in view of the small numbers present it seems unlikely that this alone affords a sufficient explanation.

## Series B

In order to determine more carefully the value of flushing with hypochlorite solution before the morning's separation, a series of tests was conducted in which flushing with 2 gallons of cold water night and morning was alternated with flushing with cold water after the evening separation and with 2 gallons of quick-acting sodium hypochlorite (50 p.p.m. av. cl.) before the morning separation. To simulate extreme summer conditions the unwashed bowl was incubated in an inverted position at 28° C. for 14 hours. From the data presented in Tables 4 and 5 it appears that the

Table 5.—Data from milk samples, series B

| Date | A.M. Samples | | | | | | P.M. Samples | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Keeping time (hrs.) | Fat % | Plate count | Coliform organisms in 1 cc. | 0.1 cc. | 0.01 cc. | Keeping time (hrs.) | Fat % | Plate count | Coliform organisms in 1 cc. | 0.1 cc. | 0.01 cc. |
| | | | | % | % | % | | | | % | % | % |
| April 23 | | | | | | | 88 | 4.2 | 900 | — | — | — |
| April 24 | 86 | 3.7 | 5,150 | — | — | — | 84 | ? | 2,150 | — | — | — |
| April 25 | 85 | ? | 3,850 | — | — | — | 85 | 3.8 | 1,950 | — | — | — |
| April 26 | 86 | 3.7 | 4,200 | + | — | — | 101 | 4.0 | 1,200 | — | — | — |
| April 27 | 81 | 3.6 | 23,400 | — | — | — | | | | | | |
| April 28 | | | | | | | 92 | 3.8 | 15,000 | — | — | — |
| April 29 | 113 | 3.6 | 6,250 | — | — | — | 113 | 4.0 | 1,850 | — | — | — |
| April 30 | 94 | 3.6 | 5,350 | — | — | — | 97 | 3.8 | 1,350 | — | — | — |
| May 1 | 109 | 3.6 | 3,350 | — | — | — | 97 | 3.8 | 2,800 | — | — | — |
| May 2 | 77 | 3.3 | 57,700 | + | + | — | 83 | 3.9 | 4,400 | — | — | — |
| May 3 | 98 | 3.2 | 5,500 | + | — | — | 83 | 4.0 | 2,850 | — | — | — |
| May 4 | 81 | 3.8 | 5,050 | — | — | — | 82 | 4.0 | 4,150 | — | — | — |
| May 5 | 109 | 3.4 | 27,100 | — | — | — | 83 | 4.0 | 1,650 | — | — | — |
| May 6 | 84 | 3.4 | 6,300 | — | — | — | | | | | | |
| Average | 91.9 | 3.54 | 7,529* | 25.0 | 8.3 | 0.0 | 90.7 | 3.94 | 2,422* | 0.0 | 0.0 | 0.0 |

* = Logarithmic average.

bacterial content of the *cream* increased considerably under both treatments, although the hypochlorite treatment reduced the contamination to less than a quarter of that from the cold water treatment and increased the keeping time from an average of 61 hours to 73·7 hours. The hypochlorite rinse was not effective in reducing the numbers of coliform organisms present, despite the known susceptibility of this class of organism to chlorine.

Comparing the evening's and morning's *milk* samples, there is no significant difference in keeping time, although the plate count and coli content of the morning's milk are definitely higher.

### Series C

In this series a study was made of the value of the cold water treatment where the separator bowl remained overnight at an average temperature of 18.2° C., varying between 12.2 and 22.2° C. Two gallons of cold water were run through the separator after the evening's and before the morning's separation. Data from seven consecutive days are presented in Table 6. In this series the keeping time of the evening's *milk* is slightly greater than that of the morning's, although perhaps no more so than would be expected in view of the lower bacterial counts. The evening's *cream* samples show a more significant advantage over the morning's, in agreement with the lower average bacterial content of the former.

### Series D

Except for the substitution of hypochlorite (50 p.p.m. av. cl.) for cold water at the morning separation, treatment in this series was identical with that in Series C. Overnight temperatures varied between 10.6 and 21.7° C. with an average value of 17.6° C. Data are presented in Table 7.

In this series the keeping time of the evening's *milk* samples exceeds that of the morning's by an average of 6.1 hours, while that of the evening's *cream* exceeds that of the morning's by 6.4 hours, indicating that the quality of the cream was unaffected by passage through a separator treated as described above. A similar comparison for Series C indicates differences of 3.4 hours for the milk, and 9.2 hours for the cream, in favour of the evening's samples. On this basis, the hypochlorite appears to be more effective than the cold water flush (Series C). Furthermore, the morning's cream in Series C shows an average count of 9,394 per cc. when separated from milk with an average count of 4,540, while in Series D, the corresponding values are 2,681 and 4,185 per cc. respectively.

### Series E

In order to obtain further confirmation of the effectiveness of the hypochlorite treatment, this was alternated with washing and steaming the separator after the evening separation. On the evening preceding the hypochlorite treatment, the bowl was flushed with 2 gallons of cold water, and remained inverted overnight at an average temperature of 18° C. (extremes of 12.2 and 25.6°). A slight change was made in the hypochlorite treatment, the strength being doubled and the quantity of rinse halved. In this manner the same amount of chlorine was passed through but the time previously required for treatment (2.5 minutes) was cut in two. Previous studies (*5*, *10*) have indicated that large amounts of dilute

TABLE 6.—DATA FROM SERIES C. SEPARATOR WASHED AND STEAMED AFTER A.M. SEPARATION; FLUSHED WITH 2 GALLONS OF COLD WATER AFTER P.M. AND AGAIN BEFORE A.M. SEPARATION

(Bowl held overnight at temperatures between 12.2 and 22° C., averaging 18.2° C.)

| Date | Milk | | | | | | | | | | | | Cream | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A.M. Samples | | | | | | P.M. Samples | | | | | | A.M. Samples | | | | | | P.M. Samples | | | | | |
| | Keeping time (hrs.) | Fat % | Plate count | Coliform organisms in | | | Keeping time (hrs.) | Fat % | Plate count | Coliform organisms in | | | Keeping time (hrs.) | Fat % | Plate count | Coliform organisms in | | | Keeping time (hrs.) | Fat % | Plate count | Coliform organisms in | | |
| | | | | 1 cc. | 0.1 cc. | 0.01 cc. | | | | 1 cc. | 0.1 cc. | 0.01 cc. | | | | 1 cc. | 0.1 cc. | 0.01 cc. | | | | 1 cc. | 0.1 cc. | 0.01 cc. |
| | | | | % | % | % | | | | % | % | % | | | | % | % | % | | | | % | % | % |
| May 6 | | | | | | | 76 | 4.0 | 3,050 | — | — | — | | | | | | | 59 | 35 | 25,500 | — | — | — |
| May 7 | 80 | 3.5 | 9,600 | — | — | — | 81 | 4.2 | 2,500 | — | — | — | 69 | 34 | 173,500 | + | + | — | 83 | 38 | 2,700 | — | — | — |
| May 8 | 86 | 3.4 | 2,300 | — | — | — | 107 | 4.0 | 2,200 | — | — | — | 70 | 32 | 5,100 | + | — | — | 97 | 36 | 1,600 | — | — | — |
| May 9 | 68 | 3.3 | 11,400 | + | — | — | 81 | 3.8 | 2,450 | — | — | — | 74 | 30 | 15,600 | — | — | — | 83 | 32 | 1,850 | — | — | — |
| May 10 | 92 | 3.6 | 3,200 | — | — | — | 89 | 4,0 | ? | — | — | — | 85 | 32 | 9,900 | — | — | — | 99 | 37 | 750 | — | — | — |
| May 11 | 85 | 3.4 | 2,800 | — | — | — | 87 | 4.0 | 2,300 | — | — | — | 92 | 30 | 3,400 | — | — | — | 83 | 32 | 1,750 | + | — | — |
| May 12 | 70 | 3.2 | 4,150 | — | — | — | 82 | 4.2 | 1,600 | — | — | — | 70 | 28 | 4,750 | — | — | — | 92 | 32 | 1,200 | — | — | — |
| May 13 | 98 | 3.4 | 4,250 | — | — | — | | | | | | | 71 | 30 | 2,950 | — | — | — | | | | | | |
| Average | 82.7 | 3.4 | 4,540* | 14.3 | 0.0 | 0.0 | 86.1 | 4.03 | 2,300* | 0.0 | 0.0 | 0.0 | 75.9 | 30.9 | 9,394* | 28.6 | 0.0 | 0.0 | 85.1 | 34.6 | 2,284* | 14.3 | 0.0 | 0.0 |

* = Logarithmic average.

TABLE 7.— DATA FROM SERIES D. SEPARATOR TREATMENT AS IN SERIES C. EXCEPT THAT NaOCl (50P.P.M. AV. Cl) SUBSTITUTED FOR COLD WATER AT A.M. SEPARATION

(Bowl held overnight at temperatures between 10.6 and 21.7° C. averaging 17.6° C.)

| Date | Milk | | | | | | | | | | Cream | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A.M. Samples | | | | | | P.M. Samples | | | | A.M. Samples | | | | | | P.M. Samples | | | |
| | Keeping time (hrs.) | Fat % | Plate count | Coliform organisms in | | | Keeping time (hrs.) | Fat % | Plate count | Coliform organisms in | Keeping time (hrs.) | Fat % | Plate count | Coliform organisms in | | | Keeping time (hrs.) | Fat % | Plate count | Coliform organisms in | | |
| | | | | 1 cc. | 0.1 cc. | 0.01 cc. | | | | 1 cc. 0.1 cc. 0.01 cc. | | | | 1 cc. | 0.1 cc. | 0.01 cc. | | | | 1 cc. | 0.1 cc. | 0.01 cc. |
| | | | | % | % | % | | | | % % % | | | | % | % | % | | | | % | % | % |
| May 13 | | | | | | | 95 | 4.4 | 2,650 | — — — | | | | | | | | | | | | |
| May 14 | 86 | 3.4 | 5,350 | — | — | — | 112 | 3.6 | 1,650 | — — — | 70 | 27 | 4,650 | + | — | — | 73 | 34 | 1,600 | — | — | — |
| May 15 | 85 | 3.0 | 5,450 | — | — | — | 87 | 3.8 | 1,250 | — — — | 73 | 26 | 4,000 | — | — | — | 86 | 32 | 1,050 | — | — | — |
| May 16 | 104 | 3.2 | 3,300 | — | — | — | 107 | 3.2 | 1,550 | — — — | 70 | 32 | 1,050 | — | — | — | 83 | 32 | 900 | — | — | — |
| May 17 | 96 | 3.0 | 4,350 | — | — | — | 96 | 3.6 | 3,100 | — — — | 74 | 30 | 2,850 | — | — | — | 85 | 35 | 650 | — | — | — |
| May 18 | 86 | 3.0 | 2,400 | — | — | — | 99 | 3.4 | 5,100 | — — — | 84 | 35 | 1,400 | — | — | — | 87 | 38 | 2,550 | — | — | — |
| May 19 | 100 | 3.2 | 5,300 | — | — | — | | | | | 84 | 34 | 4,700 | — | — | — | 79 | 33 | 1,700 | — | — | — |
| Average | 93.2 | 3.13 | 4,185* | 0.0 | 0.0 | 0.0 | 99.3 | 3.67 | 2,265* | 0.0 0.0 0.0 | 75.8 | 30.7 | 2,681* | 16.7 | 0.0 | 0.0 | 82.2 | 34.0 | 1,268 | 0.0 | 0.0 | 0.0 |

* = Logarithmic average.

TABLE 8.—DATA FROM CREAM SAMPLES, SERIES E

(Bowl held between 12.2° and 25.6°, averaging 18° C.)

| Date | Separator washed and steamed (P.M. samples) | | | | | | Separator washed and steamed (A.M. Samples) | | | | | | Separator flushed with cold water after P.M. and with NaOCl before A.M. run | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Keeping time | Fat % | Plate count | Coliform organisms in | | | Keeping time | Fat % | Plate count | Coliform organisms in | | | Keeping time | Fat % | Plate count | Coliform organisms in | | |
| | | | | 1 | 0.1 | 0.01 | | | | 1 | 0.1 | 0.01 | | | | 1 | 0.1 | 0.01 |
| | | | | % | % | % | | | | % | % | % | | | | % | % | % |
| May 19 | 86 | 33 | 4,450 | − | + | − | | | | | | | | | | | | |
| May 20 | | | | | | | 72 | 34 | 4,100 | + | − | − | | | | | | |
| May 21 | 85 | 34 | 400 | | ? | | | | | | | | | | | | | |
| May 22 | 76 | 35 | 650 | − | − | − | 86 | 38 | 2,100 | − | − | − | | | | | | |
| May 23 | 85 | 35 | 500 | + | − | − | | | | | | | 79 | 36 | 2,900 | + | − | − |
| May 24 | 75 | 35 | 34,400 | + | − | − | | | | | | | 73 | 33 | 650 | − | − | − |
| May 25 | 76 | 36 | 800 | + | + | − | 77 | 33 | 1,750 | + | − | − | | | | | | |
| May 26 | 81 | 36 | 1,500 | + | + | − | | | | | | | 91 | 40 | 4.750 | + | − | − |
| May 27 | 83 | 34 | 350 | − | − | − | 87 | 34 | 30,400 | + | − | − | | | | | | |
| May 28 | | | | | | | | | | | | | 72 | 38 | 1,450 | + | + | − |
| Average | 80.9 | 34.8 | 1,303* | 57.1 | 42.9 | 0.0 | 80.5 | 34.8 | 4,597* | 75.0 | 0.0 | 0.0 | 78.8 | 36.8 | 1,894* | 75.0 | 25.0 | 0.0 |

* = Logarithmic average.

solutions are more effective than small amounts of greater concentration, due to the influence of dilution upon the pH and the consequent narrowing in the HOCl/NaOCl ratio, and also to the mechanical removal of bacteria, but on the other hand the reduction in the time required to treat the separator is highly desirable from a practical standpoint. It was decided to discontinue these tests after May 28th as the cows whose milk was being used were removed to another farm some distance away. Data are summarized in Tables 8 and 9.

In this series the difference in keeping time between evening's and morning's milk samples has widened to 7.0 hours. The corresponding difference between evening's cream and morning's cream from the washed separator is only 0.3 hour, while with the samples from the unwashed separator treated with hypochlorite the difference is 2.0 hours. While the average bacterial count is lower for the cream samples from the hypochlorite treated separator (due to the high count from the washed separator on May 27th), comparison of the average counts of the *cream* samples with those from the corresponding *milk* samples indicates no relative difference (milk 6,273 and cream 4,597 for the washed separator, and milk 2,570 and cream 1,894 for the hypochlorite treatment).

TABLE 9.—DATA FROM MILK SAMPLES, SERIES E

| Date | A.M. Samples | | | | | | P.M. Samples | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Keeping time (hrs.) | Fat % | Plate count | Coliform organisms in 1 cc. | Coliform organisms in 0.1 cc. | Coliform organisms in 0.01 cc. | Keeping time (hrs.) | Fat % | Plate count | Coliform organisms in 1 cc. | Coliform organisms in 0.1 cc. | Coliform organisms in 0.01 cc. |
| | | | | % | % | % | | | | % | % | % |
| May 19 | | | | | | | 87 | 3.6 | 9,700 | – | – | – |
| May 20 | 77 | 3.2 | 9,200 | – | – | – | | | | | | |
| May 21 | | | | | | | 96 | 3.4 | 1,050 | ? | ? | ? |
| May 22 | 98 | 3.6 | 2,500 | – | – | – | 114 | 3.6 | 1,000 | – | – | – |
| May 23 | 88 | 3.4 | 3,600 | – | – | – | 83 | 3.6 | 900 | – | – | – |
| May 24 | 105 | 3.2 | 1,200 | – | – | – | 83 | 3.8 | 500 | – | – | – |
| May 25 | 77 | 3.3 | 1,850 | + | – | – | 93 | 3.6 | 1,450 | + | – | – |
| May 26 | 82 | 3.4 | 6,750 | + | – | – | 78 | 3.8 | 3,000 | + | + | – |
| May 27 | 91 | 3.0 | 36,200 | – | – | – | 106 | 3.6 | 900 | – | – | – |
| May 28 | 66 | 3.2 | 1,500 | + | + | – | | | | | | |
| Average | 85.5 | 3.29 | 4,027* | 37.5 | 12.5 | 0.0 | 92.5 | 3.63 | 1,433* | 28.6 | 14.3 | 0.0 |

* = Logarithmic average.

## DISCUSSION

The evaluation of the relative efficiency of the various separator treatments employed is complicated by the striking variations in the keeping time of the milk and cream, while the time-consuming nature of these studies naturally limited the number of determinations which could be made. The employment of the rate of acid production at 15.5° C. as a criterion of keeping quality is undoubtedly less accurate than the organoleptic tests employed in earlier studies (*11*), since the development of off-flavours and odours is disregarded. When these studies were commenced it was anticipated that cream from an unwashed separator bowl would sour considerably faster than the control samples from a separator properly washed

and sterilized. With certain samples of milk, however, it was found that a pronounced off-flavour could be detected twenty-four hours or more before the acidity had increased by 0.2 per cent. Nevertheless, since the majority of samples (especially cream samples) developed a clean acid flavour, the employment of the acidity increase as a criterion of keeping time appears to be justifiable, particularly in view of its greater convenience.

Since bacterial contamination of cream from an unwashed separator is serious only where considerable growth has taken place, it is not surprising to find that the temperature at which the bowl is held overnight is the most important factor influencing the degree of contamination of the cream. Where the bowl is flushed out with a sufficient quantity of cold water after skimming, and again before the morning's run after holding at approximately 18° C. overnight, the degree of contamination is surprisingly small. The value of thorough flushing is indicated by a count of 39,000,000 per cc. on cream passed through a separator, the bowl of which after flushing had stood overnight at 18° C. and was not flushed or drained before the morning run.

On the other hand, where the bowl is held at 28° C., flushing with cold water is insufficient to prevent serious contamination (Table 1, Series 2 A, and Table 4 Series B). Even where the hypochlorite is substituted for water for the morning flushing, an appreciable increase in bacterial count may be noted, although the average count is considerably below that obtained when flushing with plain cold water. (Table 4, Series B.)

It is interesting to note that in all cases where contamination from the separator was at a low level (all evening samples, and morning samples of Series A, D and E), the bacterial count of the cream is lower than that of the milk before separation. This is in agreement with the findings of Düggeli (*6*) who reported a distinct reduction in count on centrifuging clean milk. Obviously, where there are no large clumps and chains of bacteria to be broken up during centrifuging, the count should not be expected to increase, but to decrease, since a certain percentage of organisms are thrown out during the operation.

While the different series vary in the extent of the difference in keeping time between evening's and morning's cream samples, this difference in favour of the evening's cream is not due solely to the generally lower bacterial content. In Table 10, in which data are presented on samples with similar bacterial counts, it is evident that the evening samples have a significant advantage, Even after allowance is made for the higher fat content of the evening's cream. It was thought this might be due to a possibly greater contamination of the morning's samples with lactic acid bacteria from an unwashed separator, but further analysis of the data in this table indicates that a similar relative advantage holds good whether or not the separator was washed after the evening's run. A comparison of milk samples of similar bacterial content (Table 11) indicates a much narrower margin in favour of the evening's samples than is shown in Table 10.

In view of the well known fact that thick cream keeps better than thin, it is rather surprising to find that in every case but one (Series A, p.m. samples) the original milk has shown on the average a definitely longer keeping time than the cream separated from it, despite the lower bacterial

TABLE 10.—COMPARING KEEPING TIME (RATE OF ACID FORMATION) OF EVENING'S AND MORNING'S CREAM SAMPLES OF SIMILAR BACTERIAL CONTENT

| Series | Evening's cream | | | | | | Morning's cream | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Keeping time (hrs.) | Fat % | Plate count | Coliform organisms in 1 cc. | 0.1 cc. | 0.01 cc. | Keeping time (hrs.) | Fat % | Plate count | Coliform organisms in 1 cc. | 0.1 cc. | 0.01 cc. |
| | | | | % | % | % | | | | % | % | % |
| A | 94 | 48 | 1,250 | ? | ? | ? | 93 | 44 | 1,250 | ? | ? | ? |
| | 105 | 32 | 1,400 | − | − | − | 95 | 40 | 2,375 | − | − | − |
| | 78 | 34 | 2,100 | − | − | − | 74 | 30 | 2,550 | + | − | − |
| | 90 | 44 | 3,700 | + | − | − | 77 | 27 | 3,200 | − | − | − |
| C | 83 | 38 | 2,700 | − | − | − | 71** | 30 | 2,950 | − | − | − |
| D | 83 | 32 | 900 | − | − | − | 70** | 32 | 1,050 | − | − | − |
| | 73 | 34 | 1,600 | − | − | − | 84** | 35 | 1,400 | − | − | − |
| | 87 | 38 | 2,550 | − | − | − | 74** | 30 | 2,850 | − | − | − |
| E | 76 | 35 | 650 | − | − | − | 73** | 33 | 650 | − | − | − |
| | 81 | 36 | 1,500 | + | + | − | 72** | 38 | 1,450 | + | + | − |
| | 86 | 33 | 4,450 | − | + | − | 72 | 34 | 4,100 | + | − | − |
| Average | 85.1 | 36.7 | 1,786* | 20.0 | 20.0 | 0.0 | 77.7 | 33.0 | 1,897* | 30.0 | 10.0 | 0.0 |

* = Logarithmic average.
** = From unwashed separator.

TABLE 11.—COMPARING KEEPING TIME (RATE OF ACID FORMATION) OF EVENING'S AND MORNING'S MILK SAMPLES OF SIMILAR BACTERIAL CONTENT

| Series | Evening's milk | | | | | Morning's milk | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Keeping time (hrs.) | Plate count | Coliform organisms in 1 | 0.1 cc. | 0.01 | Keeping time (hrs.) | Plate count | Coliform organisms in 1 | 0.1 cc. | 0.01 |
| | | | % | % | % | | | % | % | % |
| A | 72 | 3,250 | + | + | − | 71 | 3,500 | − | − | − |
| | 83 | 4,150 | − | − | − | 86 | 3,650 | ? | ? | ? |
| B | 83 | 2,850 | − | − | − | 109 | 3,350 | − | − | − |
| | 82 | 4,150 | − | − | − | 86 | 4,200 | + | − | − |
| C | 107 | 2,200 | − | − | − | 86 | 2,300 | − | − | − |
| | 87 | 2,300 | − | − | − | 85 | 2,800 | − | − | − |
| | 76 | 3,050 | − | − | − | | | | | |
| D | 95 | 2,650 | − | − | − | 86 | 2,400 | − | − | − |
| | 96 | 3,100 | − | − | − | 104 | 3,300 | − | − | − |
| | 99 | 5,100 | − | − | − | 100 | 5,300 | − | − | − |
| | | | | | | 86 | 5,350 | − | − | − |
| E | 114 | 1,000 | − | − | − | 105 | 1,200 | − | − | − |
| | 93 | 1,450 | + | − | − | 66 | 1,500 | + | + | − |
| | 87 | 9,700 | − | − | − | 77 | 9,200 | − | − | − |
| Average | 90.3 | 2,972* | 15.4 | 7.7 | 0.0 | 88.2 | 3,162* | 16.7 | 8.3 | 0.0 |

* = Logarithmic average.

TABLE 12.—COMPARING KEEPING TIME (RATE OF ACID FORMATION) OF MILK AND CREAM SAMPLES OF SIMILAR BACTERIAL CONTENT

(Making allowance for diluting effect of butterfat in cream)

| Evening's samples | Milk samples | | | | | Cream samples | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Keeping time (hrs.) | Plate count | Coliform organisms in | | | Keeping time (hrs.) | Plate count | Coliform organisms in | | |
| | | | 1 cc. | 0.1 cc. | 0.01 cc. | | | 1 cc. | 0.1 cc. | 0.01 cc. |
| Series | | | % | % | % | | | % | % | % |
| A | 78 | 1,650 | − | − | − | 94 | 1,250 | ? | ? | ? |
| | 77 | 2,500 | ? | ? | ? | 76 | 1,500 | − | − | − |
| | 72 | 3,250 | + | + | − | 78 | 2,100 | − | − | − |
| B | 101 | 1,200 | − | − | − | 88 | 700 | − | − | − |
| | 113 | 1,850 | − | − | − | 68 | 1,200 | − | − | − |
| | 84 | 2,150 | − | − | − | 88 | 1,400 | − | − | − |
| | 97 | 2,800 | − | − | − | 67 | 1,675 | − | − | − |
| | 83 | 4,400 | − | − | − | 83 | 3,000 | − | − | − |
| C | 82 | 1,600 | − | − | − | 92 | 1,200 | − | − | − |
| | 81 | 2,450 | − | − | − | 97 | 1,600 | − | − | − |
| | 81 | 2,500 | − | − | − | 83 | 1,750 | + | − | − |
| | 76 | 3,050 | − | − | − | 83 | 1,850 | − | − | − |
| D | 87 | 1,250 | − | − | − | 83 | 900 | − | − | − |
| | 107 | 1,550 | − | − | − | 86 | 1,050 | − | − | − |
| | 95 | 2,650 | − | − | − | 73 | 1,600 | − | − | − |
| | 96 | 3,100 | − | − | − | 79 | 1,700 | − | − | − |
| E | 83 | 500 | − | − | − | 83 | 350 | − | − | − |
| | 83 | 900 | − | − | − | 76 | 650 | − | − | − |
| | 93 | 1,450 | + | − | − | 76 | 800 | + | + | − |
| | 78 | 3,000 | + | + | − | 81 | 1,500 | + | + | − |
| Average | 87.4 | 1,965* | 15.8 | 10.5 | 0.0 | 81.7 | 1,259* | 15.8 | 10.5 | 0.0 |

* = Logarithmic average.

count of the cream. The extent of this difference is clearly depicted in Table 12, where samples of evening's milk and cream are compared. Allowance has been made for the lower serum content of the cream by selecting cream samples with counts approximately two-thirds as great as that of the milk samples with which they are compared. The slower development of acid in the milk samples is evident, although individual pairs show some striking variations. Data which will appear in a subsequent paper indicate that with few exceptions the growth-rate in the cream is higher than in the corresponding milk samples during the first day of incubation at 15.5° C. Furthermore, a study of the acidity curves for both milk and cream indicates that an increase in the titration value was noted on an average around ten hours earlier with the cream than with the corresponding milk sample, although subsequently the slope of the curve is generally steeper for the milk than for the cream. This difference may result from a greater number of lactic acid bacteria in the cream, or from a more favourable growth condition in the cream than in the milk.

The possibility of cream receiving significant contamination from a well washed and steamed separator seems very remote in view of the fact

that counts on the cream are almost invariably lower than on the corresponding milk. It seems more likely that the faster growth in the cream is due to a lower lactenin content than in the milk, for it is not improbable that a portion of this growth-restraining substance is thrown out of the milk during centrifugation.

One other feature of interest is the change in the relative keeping time of the evening's and morning's milk samples during the 6 week period of these tests. Comparing the average keeping times of the milk in each series we find that the evening's samples differed from the morning's as follows:

| | | |
|---|---|---|
| Series | A | + 9.3 hours |
| | B | + 1.2 hours |
| | C | − 3.4 hours |
| | D | − 6.1 hours |
| | E | − 7.0 hours |

This suggests a definite progressive change either in the character of the milk or in the flora during this period. It cannot be due to the change from winter stabling and feed to pasture, since the cattle were not allowed out at night and were only out on pasture in the daytime during the last seven days of the period (Series E). Since coliform organisms were most abundant in the milk during the first and last series, it seems unlikely that they are a factor here. (Indeed, with the possible exception of the evening milk samples of Series A it is difficult to see any correlation between the number of coliform organisms and the rate of acidity increase.)

The rate of acid formation in the cream samples in these studies is much slower than that reported by Price, Hurd and Wilster (*16*) where cream cooled in a tank filled with water at 50° F. showed an acidity of 0.2 per cent after 42 hours, and of 0.41 per cent after 66 hours. While the initial bacterial content of their cream was probably higher than in the present studies, it seems more likely that the differences in keeping time are mainly attributable to delay in commencing the cooling process in the Oregon tests. The marked influence of delayed cooling upon keeping time has been reported by several workers (*8*, *11*) but its importance is not always appreciated by practical dairymen.

With the exception of the Metso treatment recorded in Part I, none of the treatments were found to have any appreciable effect upon the condition of the tinned surfaces of the bowl and discs.

Since these studies were commenced, a paper by Fay, Caulfield and Riddell (*7*) has appeared in which data are presented concerning the relative efficiency of hypochlorite and lye solutions, and of hypochlorite solutions of different concentrations, for sterilizing cream separators. The separators were uniformly contaminated with skim milk cultures, rinsed immediately with 3 litres of sterilizing solution, and finally with 3 litres of sterile water. The bacterial counts on this latter rinse were taken as "The relative indexes of the sterilizing action of the disinfecting solutions employed." No details are given concerning the type of sodium hypochlorite compound employed, although it has been shown that these vary tremendously in their germicidal speed (*10*). They conclude that "rinsing a separator with a chlorine solution testing approximately 200 p.p.m. gave

satisfactory results from the standpoint of the destruction of bacteria. The use of solutions originally containing 100 p.p.m. or less available chlorine is likely to lead to unsatisfactory results". In comparing these findings with those from the present studies it must be borne in mind that in their work the bowl had not been flushed out or drained after the contaminating skim milk culture had been run through, so that a great deal of the sterilizing value of the chlorine was lost through reaction with this skim-milk remaining in the bowl. The tremendous drop in chlorine concentration of the used rinse as shown in their tables confirms this, while it has been demonstrated that the sterilizing value of the used rinse is far below the value obtained by titration (*14*). Furthermore, the quantity of rinse employed in our studies was three times as great for the 50 p.p.m. and one and one-half times as great for the 100 p.p.m. solutions, as that employed by these workers, resulting in a longer contact period between the hypochlorite and the bacteria, and greater mechanical removal of the latter. Certainly the data in Table 1, Series 2 B, and in Tables 7 and 8 of the present studies fail to indicate any serious bacterial contamination following the hypochlorite treatment, although where conditions favour very active growth in the bowl between milkings (Table 4) the hypochlorite treatment may not be sufficient to prevent contamination of the cream to a significant extent.

## SUMMARY

Studies on methods of caring for farm separators indicate that the temperature at which the unwashed bowl is held is the major factor influencing the degree of contamination of cream. By flushing with two gallons of cold water after separating, holding the bowl overnight at 18° C. or lower, and again flushing with cold water before the morning run, bacterial contamination may be reduced to a low figure. Flushing with hypochlorite instead of water practically eliminates contamination. Where the bowl was held overnight at 28° C. however, the flushing treatment was insufficient to prevent serious contamination although the counts for the hypochlorite treatment were considerably lower than for cold water.

The average rate of souring of milk samples at 15.5° C. was definitely slower than that of cream of equivalent bacterial content. Cream from the evening milking also soured more slowly than that from the morning milking; a similar but less pronounced difference was found when evening's and morning's milk samples were compared. Coliform organisms appeared to exert no marked influence upon rate of acid formation at 15.5° C.

## ADDENDUM

In a recent paper, Rogers and Evans (J. Bact. 31 : 87, 1936) report that "Hand separators receiving no treatment except rinsing with water and holding submerged in a solution of tribasic sodium phosphate are physically clean and do not increase the bacterial count of the milk appreciably. The corrosive action of the alkali can be nearly or quite eliminated by the addition to the phosphate crystals of 3 per cent of sodium chromate." The addition of chromate to alkali detergents was considered by the present writer but was rejected because of its poisonous nature, and of the danger of significant quantities entering the cream.

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## Résumé


**Façons de prendre soin des écrémeuses centrifuges de ferme et leur effet sur la qualité de la crème, par C. K. Johns, Ferme expérimentale centrale, Ottawa, Ontario.**

Une étude des façons de prendre soin des écrémeuses centrifuges de ferme indique que la température à laquelle le bol non lavé est tenu est le facteur qui influe le plus sur le degré de contamination de la crème. Le lavage du bol avec deux gallons d'eau froide après l'écrémage, le maintien du bol toute la nuit à une température d'environ 18° C. et un relavage à l'eau froide avant la traite du matin, réduisent la contamination bactérienne à une faible proportion. Le lavage avec une solution d'hypochlorite au lieu d'eau supprime presque complètement la contamination. Cependant, lorsque le bol est tenu toute la nuit à une température de 28° C., ce lavage est insuffisant pour prévenir une grave contamination, mais même dans ce cas, la numération après le traitement à l'hypochlorite est beaucoup plus faible qu'après le traitement à l'eau froide.

A une température de 15.5° C. les échantillons de lait ont suri en moyenne beaucoup plus lentement que la crème ayant un contenu égal de bactéries. De même, la crème de la traite du soir a suri plus lentement que celle provenant de la traite du matin. Une différence semblable, mais moins prononcée, a été constatée entre les échantillons du lait du soir et du matin. A 15.5° C. les organismes coliformes ne paraissent exercer aucun effet sensible sur la rapidité de la formation de l'acide.

# NOTES ON THE SURVIVAL OF THE EGGS AND FREE-LIVING LARVAE OF SCLEROSTOMES ON PASTURE[1]

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[Received for publication February 1, 1936]

The bursate nematodes of horses and sheep form one of the major sources of loss to owners of these animals. This group of worms includes the Sclerostomes (Red or Pallisade worms) of horses, the hookworms, "twisted" stomach worms and a number of smaller forms in sheep, and it is difficult to find a single animal of either species which is completely free from them. They all have a similar life-cycle, although no two are quite identical. Minute thin-shelled eggs are passed to the exterior in the animal's droppings. When the temperature is above 10° C. these eggs develop small larvae, which immediately they are completely developed, leave the shell and feed actively on bacteria and so on in or near the droppings. These larvae are called the first-stage larvae and although they can develop at the temperature mentioned, this must be regarded as being near the minimum. The optimum temperature is, for most species, around 25° C. to 27° C., when hatching takes place within 24 hours. The first stage larvae grow rapidly at this temperature and ultimately moult, after which they are called the second-stage larvae and they also feed, grow and moult. On this occasion however, the moult is incomplete and the cast cuticle is retained as a protective sheath. The third stage larvae are no longer able to feed and they must live on material stored in their own bodies as the result of their previous feedings. They can live for a considerable length of time. The third stage larvae are the only ones capable of infecting a new host, and they are referred to as the *infective larvae:* for the purpose of this paper the two previous stages are collectively referred to as *free-feeding larvae.* Under the most favourable conditions, the infective stage is not reached in under five days: in practice this period is almost always exceeded.

It is a universal custom to run foals with their dams, and a frequent custom to run lambs with the ewes, on pasture, and as young stock are more susceptible to worm damage than adults, which act as distributors of worm eggs, it is extremely important to find methods of controlling infections on grassland, and nowhere is it more difficult to do so.

## PHYSICAL TESTS

On Montreal Island, where these observations were made, the average mean temperature is about −15° C. in January and 21° C. in July, and daily fluctuations of temperature may be considerable. In summer heavy thunderstorms are common.

During the summer of 1934, from the end of June to September, 20 gram cultures of fresh horse faeces were put on pasture in bottomless frames, 12 inches × $5\frac{3}{4}$ inches, and treated with artificial fertilizers, urine and copper sulphate to determine whether any of these substances might be used to kill eggs and free-feeding larvae on grassland. These experiments failed, as it was found that if the faeces, even in the untreated plots,

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were well spread either by hand or by heavy rain, few, if any, infective larvae could be recovered from faeces, grass or soil, even when the plots were under the shade of trees; moreover, such larvae as were isolated from small lumps were comparatively inactive.

To indicate the cause or causes of this inability of the larvae to reach the infective stage, a series of plots of the same size was started at the end of July, 1934. There were four groups of four plots each. One group was exposed to the sun all day; the larvae from this group were isolated after nine days. Another group received the sun in the early morning and evening, another only in the morning and the fourth was not exposed to the sun at all; the larvae in these three groups were isolated after 13 days.

In each group of four plots, the faeces in one were spread and kept dry; in another they were spread and moistened; on the other two they were not spread, but were moistened on one and kept dry on the other.

The four plots on which the faeces were spread and kept dry yielded no larvae.

The four plots on which the faeces were spread and moistened yielded a few larvae (0 to 340).

The four plots on which the faeces were not spread and kept dry yielded more (200 to 5,750).

The four plots on which the faeces were not spread and moistened yielded the most larvae (800 to 5,750).

The two plots yielding 5,750, were from the group exposed to the early morning sun, and the lowest numbers (0 to 800), were from the group which received no sun.

These plots and the failures with the controls for the chemically treated plots mentioned above suggest that complete spreading of faeces must probably be considered as harmful to the free-living stages of Sclerostomes under pseudo-tropical summer conditions. It has been shown that in South Africa sunlight is very lethal to *Haemonchus contortus* infective larvae (*22*) but they, unlike the Sclerostome larvae, are almost non-resistant to desiccation.

Summer conditions having been found to be lethal to spread horse faeces, plots were made on December 13, 1934, to test the effects of winter conditions. Six plots with varying quantities of faeces—10 to 400 grams—were put directly on the ground in heaps, so that the heavier the weight of faeces put in the plot, the larger the proportion of faeces in the centre of the heap and therefore, self-protected. These plots were lifted in the spring and cultured.

| | | |
|---|---|---|
| 10 gms. of faeces | yielded | 300 infective larvae. |
| 20 gms. of faeces | yielded | 1,500 infective larvae. |
| 50 gms. of faeces | yielded | 11,000 infective larvae. |
| 100 gms. of faeces | yielded | 9,600 infective larvae. |
| 200 gms. of faeces | yielded | 10,600 infective larvae. |
| 400 gms. of faeces | yielded | 15,500 infective larvae. |

Twenty-one 40-gram cultures were put in glass jars. Fourteen of these were open, seven protected from the sun—where the temperature would reach the minimum without violent fluctuations—and seven unprotected. The seven cultures in closed glass jars were in the sun to be

subjected to the maximum amount of temperature variation. Three of these jars, one of each series, were put out immediately after the faeces were passed. The others were kept in a constant temperature room at 25° C. (± 2° C.) and then exposed at varying intervals as shown in Table 1, to test the effect on the different free-living stages.

On April 1, all cultures were put in the constant temperature room again until April 11, to give any undeveloped larvae time to reach the infective stage.

Some eggs, for unknown causes, are slow to embryonate and hatch, and it is probable that some had not hatched when the cultures, which were kept in the constant temperature room for 24 to 96 hours, were put out of doors. Therefore, it is probable the cultures which were intended to test the resistance to cold of free-feeding larvae also contained some eggs.

TABLE 1.—NUMBER OF INFECTIVE LARVAE OBTAINED FROM 40 GRAM CULTURES PUT OUT OF DOORS IN WINTER FOR THREE AND A HALF MONTHS AND SUBSEQUENTLY RECULTURED INDOORS AT 25° C. (± 2°C.) FOR 11 DAYS

| Hours developed at 25° C. (± 2° C.) before being put out of doors | In open glass jar | | In closed glass jar |
|---|---|---|---|
| | In shade | In sun | In sun |
| 0 | 9,500 | 8,900 | 0 |
| 24 | 0 | 25 | 50 |
| 48 | 225 | 100 | 5 |
| 72 | 550 | 125 | 5 |
| 96 | 650 | 125 | 0 |
| 120 | 2,700 | 2,400 | 150 |
| 480 | 32,200 | 11,000 | 0 |

Controls, kept in constant temperature room all winter, 10,000; 40,500 and 64,000.

These experiments can be interpreted as indicating the great resistance of infective larvae and of eggs, but not of free-feeding larvae, to cold; and the susceptibility of all free-living stages to violent and frequent fluctuations in temperature. In Montana, where the winter temperature also falls below −17° C., infective larvae in horse faeces have survived for three and a half years (*21, b, c, d, e,*) while in Germany it has been shown that alternate thawing and freezing is rapidly lethal, while continuous freezing is not (*5, 6, 12, 14*).

Sheep faeces were treated in a similar manner to the horse faeces but no larvae were obtained from any put outside, although the controls kept in the constant temperature room all winter, yielded some larvae. In South Africa it has been shown that the infective larvae, but not the eggs, of *Haemonchus contortus* (which is not the hardiest species of sheep nematode) withstand 0° C. for several months (*22*), and it has been stated that the infective larvae will withstand the Canadian winter.

The Trichostrongyles of sheep are highly resistant to drought in the embryonated egg and infective larval stages, but not in the other free-living stages (*20*).

## CHEMICAL TESTS

During the summer of 1934 some plots were made on grass to test the lethal effects of urine and chemicals on Sclerostome larvae (*15, 16*).

The first series was put out on May 23, using faeces four days old in which the Sclerostome larvae had been encouraged to develop; a control showed that they had developed well. Eighteen 40-gram lots of these

faeces were put in piles on freshly mown grass in bottomless and topless wooden frames one foot square. Ten days later the faeces were spread and treated as shown in Table 2, and five days later half of each plot was removed and the larvae isolated; the numbers of larvae obtained with the chemicals tested, are also shown in the table. After a further four days the same quantities of chemicals were sprayed on to the remaining half-plots, *i.e.*, at double the previous quantity per acre. The re-treated half plots therefore received 5 cc. plus 10 cc. or 10 cc. plus 20 cc., 1 gram plus 2 grams or 1.5 grams plus 3.0 grams as the case may be. (One gram per square foot is at the rate of slightly under one short hundredweight per acre, and 10 cc. is equivalent to slightly under 100 imperial gallons per acre.)

TABLE 2.—LARVAE OBTAINED FROM 20 GRAMS OF FAECES ON GRASS TREATED WITH:

| | Treated once: | | Treated twice: | |
|---|---|---|---|---|
| | 5 cc. | 10 cc. | 5 + 10 cc. | 10 + 20 cc. |
| Urine, fresh, horse | 800 | 350 | 170 | 100 |
| Urine, stale, horse | 1,000 | 750 | 125 | 170 |
| Copper sulphate 1% | 650 | 250 | 330 | 200 |
| Copper sulphate 2% | 325 | 2,800 | 130 | 300 |
| | 1 gm. in 4 cc. water | 1.5 gms. in 9 cc. water | 1 + 2 gms. in 4 + 8 cc. water | 1.5 + 3 gms. in 9 + 18 cc. water |
| Urea | 500 | 1,050 | 160 | 225 |
| Nitrate of soda | 600 | 400 | 80 | 180 |
| Sulphate of ammonia | 350 | 500 | 100 | 200 |
| Muriate of potash | 300 | 180 | 90 | 200 |
| Controls—untreated | 1,200 and 600 | | 50 and 275 | |
| | While some of these larvae were extended the majority were alive | | Fewer larvae active | |

During the summer of 1935 a few more plots were made to test the effects of artificial fertilizers, etc., on the eggs, free-feeding larvae and infective larvae of Sclerostomes.

At the end of May faeces were collected from the usual stable and were cultured on a tray in the dark constant temperature room at 25° C. (± 2° C.) until July 25, when 25 grams of faeces (which was equivalent to approximately 40 grams of fresh faeces) were put into bottomless and topless wooden boxes 12 inches by $5\frac{3}{4}$ inches at the north side of the Institute, sheltered from the sun and heavy rain.

Each plot was treated with 15 grams of dry fertilizer. The fertilizers tested and the numbers of larvae obtained on isolating them from the grass and faeces a week later are shown in Table 3 (third column).

On August 7, 1935, fresh faeces were collected and immediately put in small lumps on grass at the rate of 40 grams per 12 inches by $5\frac{3}{4}$ inches plot. One series was treated with 15 grams per plot of the various fertil-

TABLE 3.—NUMBERS OF LARVAE OBTAINED FROM GRASS PLOTS WHEN TREATED WITH:

| 15 grams of | Treated Aug. 7–35 With the majority of the larvae in the Egg state | Treated Aug. 9 With the majority of the larvae in the Free-feeding larval stage | Treated July 25 With the majority of the larvae in the Infective larval stage |
|---|---|---|---|
| Urea | 1 a | 10 c | 800 b |
| Calurea | 0 | 25 c | 4,500 c |
| Cyanamid-powdered | 0 | 10 c | 1,000 a |
| Calcium nitrate | 1 a | 10 c | 4,100 |
| Calnitro | 0 | 50 b | 8,200 |
| Nitro-chalk | 0 | 50 | 6,600 |
| Sodium nitrate | 0 | 2 c | 20,600 |
| Potassium nitrate | 0 | 20 b | 9,300 |
| Ammonium sulphate | 0 | 0 | 9,200 c |
| Ammonium chloride | 0 | 5 | 7,500 b |
| Ammonium carbonate | 1 | 2 | 3,250 c |
| Diammonium phosphate | 1 | 25 b | 11,800 c |
| Carbonate of potash | 100 b | 1 | 3,300 b |
| Sulphate of potash | 10 a | 150 | 11,600 c |
| Muriate of potash | 20 a | 75 a | 6,000 |
| Kainit | 50 b | 20 a | 8,900 c |
| 20% superphosphate | 4 a | 75 a | 2,400 |
| Hydrated lime | 100 b | 250 b | 3,000 a |
| Calcium hypochloride | 0 | 10 a | 4,900 c |
| Paradichlorbenzol | 150 c | 50 a | 11,100 |
| Copper sulphate | 0 | 25 a | — |
| Untreated (controls) | 100 b | 900 b | 12,600 |
| | 20 b | 200 b | 6,700 |
| | 50 c | 100 a | |

a—majority or all dead.
b—many dead.
c—a few dead.
No mark—majority of larvae active.

izers at once and another with the fertilizers 48 hours later. Table 3 (1st and 2nd columns) shows the fertilizers used and the numbers of larvae isolated from the various plots after four and a half weeks exposure. These plots also were in a sheltered situation on the north side of the Institute.

Many of the fertilizers at this rate (1½ short tons per acre) damaged the grass considerably. They can be divided into three groups:

*(a)* Those which almost destroyed grass or clover, *i.e.*, carbonate of potash, Kainit, calcium hypochloride and all the nitrogenous fertilizers tested, with the exception of calcium nitrate, Calnitro and Nitro chalk; the latter did slightly less damage.

*(b)* Copper sulphate and muriate of potash which did some damage, and

*(c)* Sulphate of potash. 20% superphosphate and hydrated lime which did least damage.

In small quantities there would be a tendency for many fertilizers, such as calcium nitrate, Kainit and, to a less degree, Calnitro and Nitro chalk, to roll from off hard lumps of faeces.

Again the low number of infective larvae obtained from the untreated plots—only a hundredth of the number obtained when the faeces are developed in the laboratory—detract largely from the value of the figures

obtained for the plots treated when the parasites were in the egg and free-feeding stages. Furthermore, 15 grams of chemical to treat 40 grams of faeces is extremely high. It has been found that in similar sized cultures in glass jars indoors, only 0.075 gram of Paradichlorbenzol, 0.33 gram of Urea, and quantities very considerably under 15 grams of the other fertilizers, prevent development of larvae to the infective stages, when the faeces were treated before the eggs can hatch.

It is impossible to know how many of the larvae which were isolated from the faeces treated after the infective larvae had developed, would have been capable of causing infection. Even those cultures in which the majority or many appeared dead, must be considered potentially dangerous because, in the process of isolation, the larvae had to swim from the grass and faeces through the enclosing cheese cloth; this may have little significance however, as it has been shown that larvae treated with copper sulphate may die after removal from a 1% solution, although immediately washed (*21d*). In Europe, some fertilizers and copper sulphate have been tested on eggs and larvae in faeces and free; some have shown lethal properties, but none is sufficiently rapid to destroy larvae on grass (*5*, *13*, *18*, *19*).

These experiments show the difficulties of the problem and suggest that until some more efficient chemical is found, mechanical means—such as the frequent removal of the bulk of the faeces, followed by chain harrowing—offer the most reliable, although extremely laborious, means of keeping pastures clean under climatic conditions similar to those of summer on the Island of Montreal. When larvae are in a suitable environment, such as soil, through which they can migrate, they can be protected from climatic conditions by plowing. This may concentrate sheep infective larvae (*20*) and produce subsequent outbreaks of disease in the hosts. Moreover, it has been shown that, except in very heavy soils, burial offers no great obstacle to the return to the surface of human hookworm larvae (*1*, *2*, *3*, *4*, *10*, *17*) Similarly, plowing does not kill larvae of horse Strongylostomes (*21 d*) although it has been shown that when larvae are buried in coarse sand or heavy clay (*21 f*, *h*,) few migrate vertically and reach the surface, but 3.5% may do so in sandy clay (*21 h*) and smaller percentages in other soils (*21 f*, *g*, *h*).

Bleaching powder (*9*) and copper sulphate (*7*) have been suggested as possible chemicals to kill larvae on grassland, and lime on pastures contaminated with the dog hookworm larvae (*11*).

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## Résumé

**Notes sur la survivance des œufs et des larves de sclerostomes sur le pâturage, par I. W. Parnell, Institut de Parasitologie, Collège Macdonald, Qué.**

L'auteur souligne l'importance qu'il y aurait de trouver le moyen de détruire les larves des vers intestinaux des animaux domestiques sur les pâturages. Des expériences préliminaires indiquent que beaucoup d'engrais chimiques, que l'on sait avoir un effet mortel sur les larves dans les conditions de laboratoire, n'ont pas le même effet lorsque les larves sont sur le gazon, et que dans les conditions d'été pseudo-tropicales le taux de mortalité est plus élevé que pendant la période des grands froids.

# THE EVALUATION OF VITAMIN-A SUPPLEMENTS BY SPECTROMETRIC METHODS[1]

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[Received for Publication February 15 1936.]

Improvement in the quality of supplementary feeding stuffs through legislative control has been one of the aims of the agricultural industry. The need for an extension of such control to include fish liver oils is too evident, particularly to those concerned with the production of poultry and swine, to require further emphasis here. The Feeding Stuffs Act does not, and up to the present could not, provide for the standardization of these products, at least so far as their vitamin-A content is concerned. This situation, unsatisfactory as it was known to be, continued to exist because biological methods alone were available for the determination of Vitamin-A. Biological assay is an expensive, time-consuming process and yields at best only approximate values. Obviously the solution of the problem lies in the elaboration of chemical or physical methods of analysis.

The last decade has seen phenomenal advances in our knowledge of the chemical nature of vitamins. Among these was the discovery of the chemical identity of vitamin-A. Highly purified concentrates were prepared from liver oils, by means of which certain chemical and physical properties of vitamin-A were established. The foundation was thus laid for the development of new methods for its estimation. A physical method has been developed upon the observation that vitamin-A absorbs radiations in the ultra-violet region of the spectrum.

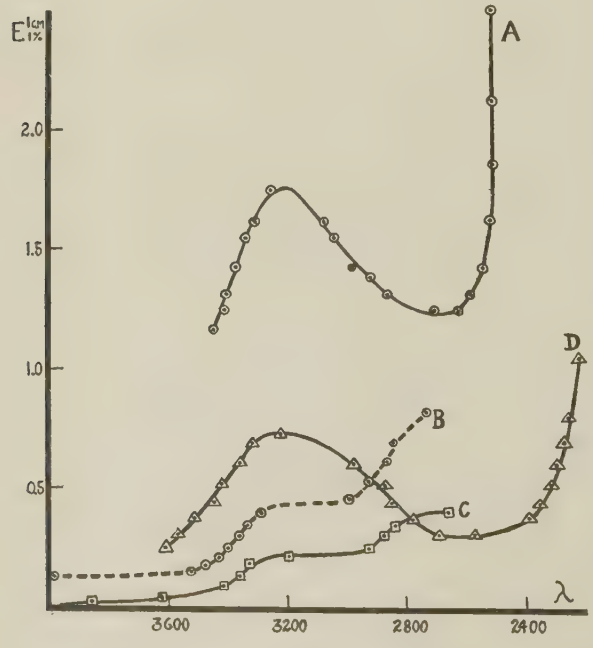


FIGURE 1.—A. Cod-liver oil—medicinal. B. Pilchard oil. C. Pilchard oil—saponified. D. Poultry cod-liver oil—saponified.

Light is absorbed by a substance in virtue of its particular chemical constitution or, as the physicist would express it, because the periods of vibration of its molecules correspond with those of the light absorbed. The absorption of light by the substance may be "general" or it may be "selective"

[1] Excerpt from an address "Biochemistry and Agricultural Practice", by W. D. McFarlane before the Northern Alberta Branch C.S.T.A., February 5, 1936.

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in that certain wave-lengths are absorbed with particular intensity so that a graph of the percentage of light absorbed plotted against wave-length would show maxima and minima. Such a graph represents the spectrum of the substance. Two substances with the same spectra are identical substances. Once the absorption spectrum of a substance has been established and it is known that solutions of the substance obey certain laws, the amount of the substance in solution may be determined by absorption measurements.

In 1928 Heilbron and Morton (*1*) found that vitamin-A is characterized by a broad intense absorption band with a maximum near 328 m.$\mu$. An absorption curve of vitamin-A obtained by us with a sample of medicinal cod-liver oil is illustrated in Figure 1 (curve A). Subsequent work (*2*, *3*, *4*, *5*) has dealt particularly with the selective absorption shown in the ultra-violet by products containing vitamin-A, and with the correlation of spectroscopic and biological data on a quantitative basis. The conclusion arrived at in 1934 by the Vitamin Standardization Committee of the League of Nations is that "within certain definite conditions, measurements of the coefficient of absorption (E) at 328 m$\mu$ affords a reliable method for measuring the vitamin-A content of liver oils and concentrates." The physical method of assay is being widely applied to medicinal oils. If the "certain definite conditions" are being complied with in the analysis of liver oils of cattle and poultry grade, then a rapid and precise method, perfectly adapted for routine agricultural work, is available for the estimation of vitamin-A.

## The Determination of Vitamin-A in Crude Oils by the Physical Method

In this department an investigation has been made of the vitamin-A content of medicinal cod-liver oils on sale in Alberta. This investigation was later extended to include representative samples of cattle and poultry oils and to determine whether any special difficulties arose in their analysis by the spectrophotometric method. The results of this paricular phase of the work are presented in Table I. The details of the apparatus[4] and procedure used will appear elsewhere in a paper covering the entire investigation.

Attention has been drawn (*6*, *3*) to the possibility that substances other than vitamin-A, which absorb in the near ultra-violet, may be present in crude oils. In crude oils the 328 m$\mu$ band may be poorly defined due to: (a) the presence of pigments with absorption bands in the visible spectrum, but with end absorption estending in the ultra violet beyond 328 m$\mu$; and (b) to the presence of free unsaturated fatty acids absorbing intensely further in the ultra-violet. The type of absorption which results under these conditions is illustrated in Figure 1 (Curve B). The League of Nations Report (*7*) requires that the method be applied only to the non-saponifiable fraction of medicinal oils. More recent observations (*8*) question the necessity of saponifying the oil.

It is evident (Table 1) that the results obtained by direct spectrophotometric measurements on crude oils, are erroneous. The non-saponifiable

[4] Bellingham and Stanley ultra-violet quartz spectrograph fitted with a B. and S. short-focus rotating sector.

TABLE 1.—THE APPARENT VITAMIN A CONTENT OF FISH LIVER OILS OF CATTLE AND POULTRY GRADE AS CALCULATED FROM SPECTROPHOTOMETRIC DATA

| Oils used | Free fatty acids % | Vitamin A. (I. U.[1]) | |
|---|---|---|---|
| | | Oil untreated | Oil saponified |
| A. Cod-liver oils | | | |
| 1. Norwegian | 5.10 | 1120 | 730 |
| 2. Norwegian* | 0.78 | 416 | 268 |
| 3. Nova Scotian | 0.14 | 1110 | 1018 |
| 4. Nova Scotian | 0.63 | 1200 | — |
| 5. Newfoundland | 0.96 | 1920 | 1720 |
| 6. Source ?* | 1.13 | 352 | 278 |
| 7. Source ?* | 3.18 | 656 | 524 |
| B. Pilchard oils* | | | |
| 1. | 0.75 | 680 | 248 |
| 2. | 0.70 | 352 | 278 |
| 3. | 0.88 | 560 | 354 |
| 3. Pilchard and Halibut oil blend | 0.90 | 2240 | — |

[1] International Units:— Observed extinction coefficient $\left[E\ \frac{1\%}{1\ cm.}\right]$ multiplied by 1600. Calculated on assumption that pure vitamin A has a value of $E\ \frac{1\ cm.}{1\%} = 1600$. [Carr and Jewell (16)].

fractions prepared by the official procedure (*7*) always yield very much lower results. Oils of relatively low vitamin-A content (Table 1, asterisk) did not give a typical vitamin-A absorption band, but only a marked deflection in the curve in the region of 328 m$\mu$. The absorption band is masked by some substance(s) still contained in the non-saponifiable fraction of these oils which absorbs strongly in the vicinity of 280-300 m$\mu$ (Figure 1, curve C). Heilbron, Gillam and Morton (*5*) have similarly observed that in crude oils the absorption band shows poor persistence, due to the presence of unidentified substances which were not eliminated by saponification.

It is therefore doubtful whether the values found for these particular oils (Table 1, asterisk) represents their true vitamin-A content. It is certain that the amount of vitamin-A in these oils does not exceed the values given.

## The Relation of Spectroscopic to Biological Methods of Assay

The efficacy of carotene as a substitute for vitamin-A in the diet of the rat and of the chick is well known. The transformation of dietary carotene into vitamin-A is believed to take place in the liver. It is now generally conceded that vitamin-A, as such, does not occur in the plant kingdom. Grasses, legumes, carrots, etc., are relatively rich sources of vitamin-A, by virtue of their high carotenoid content. The provitamin activity of carotenoids other than carotene is not so definitely established. The vitamin-A activity of yellow corn appears to be due, not to carotene itself, but to a related pigment, kryptoxanthine (*9*). The carotenoids of butter, egg-yolk and cow's blood serum include kryptoxantine as well as carotene and xanthophyll (*10*, *11*, *12*). The latter is biologically inactive for the rat but may be active for the chick (*18*, *19*, *20*).

Biological assay measures the total growth-promoting effect due to the sum of the contributions of vitamin-A and that fraction of the carotene and kryptoxanthine which is transformed into vitamin-A in the rat. The vitamin-A, carotene and kryptoxanthine content of butter and egg-yolk can be determined by methods devised mainly by Gillam and co-workers (*13*, *10*, *11*, *12*). These methods involve photometric analysis, in the visible and ultra-violet, of the fractions resulting from a phase-separation of the non-saponifiable fraction. The procedure is of very recent origin so that no information relating the results to those obtained by biological assay (rat units) is yet available. The indications are that the relationship once established may not hold for species other than the rat. Carotenoid pigments are evidently not transformed into vitamin-A by different species with equal facility. For carotene, according to Ahmed and Malik (*14*), the conversion activities of different species are, rats 100, chickens 24, rabbits 16, and pigeons 12.

The evaluation of vitamin-A supplements of plant origin by other than actual feeding trials will be possible only when we are more fully informed in regard to the vitamin-A value of the various carotinoids for farm animals. The analytical methods devised by Gillam et al. should, however, be a valuable aid in researches on the preservation of the provitamin-A content of grasses and legumes during harvesting in so far as they make possible the rapid estimation of the carotinoids known to have provitamin A activity. At some future time they may be applied in the control of the quality of commercial alfalfa products.

The carotenoid content of refined cod-liver oils is too small relative to the vitamin-A content to exert any appreciable influence on the results of spectroscopic and biological analysis. Refined pilchard oils are, however, highly colored by a golden brown pigment. *The vitamin-A content of pilchard oil relative to a good grade poultry cod-liver oil as recorded in Table I is not in accord with the results of feeding trials with chicks as carried out by Gutteridge (15) and by others.*

There can be no doubt that the spectrophotometric data at least establishes the maximum amount of true vitamin-A in pilchard oil. The question naturally arises as to how far the pigment content of this oil (assuming the pigment has provitamin activity for the chick) would account for the discrepancy in results. Although our observations in this regard have not yet reached a stage which would permit a definite pronouncement being made at this time, they do indicate that to account entirely for Gutteridge's results we must postulate the existence of a substance in pilchard oil which promotes growth in the chick but is non-pigmented and does not absorb at 328 mμ.

Shrum and How (*21*) have made absorption measurements on the non-saponifiable fraction of two samples of pilchard oil. Their values for the vitamin-A content of this oil are of the same order as those recorded in Table I. By biological test (rat units) however, the vitamin-A content of the oil was only about 50% of that obtained by the spectroscopic method. When they calculated the vitamin-A from the difference in the absorption coefficients (non-saponifiable fraction) obtained before and after destruction of vitamin-A by aeration of the oil at 90° C., the results agreed very well with those of the rat assay.

## SUMMARY AND CONCLUSIONS

The literature pertaining to the problem of evolving a routine procedure for determining the vitamin and provitamin-A content of feeding stuffs has been reviewed. We have intentionally omitted any reference to the chemical method (antimony trichloride test) because we believe the physical method offers much greater possibilities in this regard. Some results of vitamin-A determinations on fish-liver oils obtained with a quartz spectrograph are presented. They indicate:—

(1) That cod-liver oils of poultry and cattle grade sold in Alberta vary widely in their vitamin-A content.

(2) Provided the vitamin-A content of the oil is not exceptionally low, there is no reason to doubt that the spectrophotometric method applied to the non-saponifiable fraction of poultry oils gives accurate results. So far as the better grade poultry oils are concerned, the absorption measurements have been quite as satisfactory as those with medicinal oils.

(3) With very low grade oils the results obtained may be still too high.

In the case of pilchard oils the conflict between the results of the physical measurements recorded herein and the biological evaluation made by others indicates the need for further investigation on this particular oil.

We have concluded from a study of medicinal oils that the only safeguard the purchaser has is the reputation and integrity of the manufacturer. Medicinal oils produced by well established firms generally conform to the standards of a good cod-liver oil. Although a much smaller number of cod-liver oils of feed grade have been included in this study it would appear that a similar conclusion is warranted. Obviously cod-liver oils should be required to conform to some standard. Since the majority of the oils on sale for feeding purposes are not actually "crude" preparation, many being of the near-medicinal class, and since the use of sun-rotted oils is being discouraged (*17*) we can see no reason (unless it be the expense of the equipment) why the physical method of analysis should not be used to establish and enforce a standard, so far as vitamin-A content is concerned.

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## Résumé

**L'évaluation des suppléments de vitamine-A par des méthodes spectrométriques, par W. D. McFarlane et L. Rudolph, Université d'Alberta, Edmonton, Alberta.**

Le contenu en vitamines-A de quelques marques ordinaires d'huile de foie de morue et d'huile de pilchard, destinées aux bovins et aux volailles et vendues en Alberta, est donné. Les résultats sont calculés d'après des mesures d'absorption, effectuées sur la fraction non saponifiable de l'huile avec un spectrographe de quartz. Le contenu en vitamines-A de ces huiles variait de 248 à 1720 unités internationales. Les ouvrages traitant du problème de l'élaboration d'un procédé de routine pour la détermination du contenu de la quantité de vitamines-A, et de la provitamine-A, sont passés en revue.

# EFFECT OF VERNALIZATION ON THE INCIDENCE OF LOOSE SMUT IN WHEAT[1]

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[Received for publication February 11, 1936]

Environmental conditions, such as temperature, humidity, soil moisture, soil aeration, and depth of planting exert a pronounced influence upon the development of the smut fungi. During spore germination and penetration of the host tissue, the action of environment on both parasite and host plant is direct or primary. Many familiar instances of the direct effect of environment on smut infection might be cited. Thus, for example, heavy infection of wheat seedlings by the bunt fungi occurs only at relatively low temperatures (10° to 15° C.), and at temperatures above 20° C. practically no infection takes place. Similarly, it has been found by Tapke (*9*) that the loose smuts of wheat and barley require a high atmospheric humidity for successful floral infection.

From the time the smut fungus gains entrance to its host until spore formation takes place, changes in environment can only affect the development of the smut in an indirect or secondary manner by altering the physiology of the host plant. About the secondary effects of environment on smut development comparatively little is known. Smith (*7*) found that Hope wheat, which is normally resistant to bunt, became susceptible when grown continuously in a cool environment. In experiments conducted by Taylor and Zehner (*10*), deep seeding of winter barley gave a higher percentage of plants affected with loose smut than did shallow seeding. Leukel (*4*) in summarizing his experiments with loose smut of barley states that "A relatively high soil temperature before emergence seems to favour loose-smut development more than does a low soil temperature." However, in view of more recent work, it is possible that the seed used by Taylor and Zehner and by Leukel was infected with *Ustilago medians* Bied. as well as with *U. nuda* (Jens.) Rostr. Since *U. medians* infects barley seedlings in much the same manner as does the covered smut, *U. Hordei* (Pers.) K. and S., the effects observed by these workers may have been due to the direct action of environment on spore-germination and infection, rather than to its indirect action on the development of the smut within the host plant.

There appears to be some disagreement among different workers as to the effect of environmental conditions on the incidence of loose smut of wheat in plants grown from infected seed. Fromme (*1*) found that seed sown in rich soil produced a lower percentage of smutted heads than seed sown in poor soil. Similar results were obtained by Gassner and Kirchhoff (*2*), who showed that the incidence of loose smut was somewhat higher in wheat and barley growing on a light clay-sand soil than on a heavy soil. They also found that the percentage of loose smut was lower in plots which had received a complete fertilizer than in those which had received

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a fertilizer lacking one or more of the main constituents. Another conclusion reached by them was that seed germinated at low temperatures produced fewer smutted plants than seed germinated at high temperatures. Hiltner and Lang (*3*), however, found that fertilization of wheat with calcium cyanamide had no effect on the percentage of loose smut in the crop, and Tapke (*8*), after growing several thousands of hand-inoculated seeds in fertilized and in unfertilized plots, came to a similar conclusion. In a more recent paper Neill (*5*) states that the incidence of loose smut of wheat appeared to be unaffected by external conditions, such as temperature, rainfall, or date of sowing, and to depend solely upon the amount of infection present in the seed.

The recent work of Lyssenko and his associates (*12*) on vernalization, and the application of this process in Russia to practical agriculture has directed attention to the problem of controlling smut in vernalized seed. In the vernalization of wheat it is necessary to hold the germinated seed for a considerable time at a low temperature. This procedure might be expected to affect the germination of the smut spores, and also to influence the process of infection and the further development of the mycelium within the tissues of the host.

Nemlienko (*6*) studied the effect of vernalization on infection of wheat by bunt, *Tilletia Tritici* (Bjerk.) Wint., and found that vernalized seed produced less than half as many bunted plants as unvernalized seed. Seed inoculated after vernalization gave practically the same percentage of bunt as seed inoculated before vernalization. This result led Nemlienko to conclude that bunt infection occurred after seeding and not at the time of vernalization.

## METHODS

In the spring of 1934 the writer undertook an experiment to determine the effect of vernalization on the incidence of loose smut in spring wheat. Seed of the variety Marquis, artificially inoculated with loose smut, was used.

Before vernalization the seed was treated with 0.25% Semesan solution to prevent the growth of moulds. It was then placed in Erlenmeyer flasks, and distilled water was added to bring its moisture content up to about 50%. The moistened seed was kept at room temperature until germination had begun, when it was transferred to a refrigerator and there held at 3° C. for 15 days.

One lot of treated seed was sown in the field immediately after being removed from the refrigerator. A second lot, vernalized in the same way, was dried before being sown. As a control, a third lot of seed was sown on the same date without vernalization. The seeds were spaced $2\frac{1}{2}$ inches apart in rows.

## RESULTS

Vernalization did not appear to accelerate the development of the plants grown from the spaced seeds. It should be pointed out, however, that these plants tillered profusely, and that many of the heads did not ripen. In such material it might be expected that a difference of two or three days in the time of heading and ripening would pass unnoted.

Three rows of uninfected Marquis wheat were drilled in at the usual rate of seeding alongside the rows of spaced plants. The seed sown in two of these rows was vernalized in the same way as the infected seed already referred to; one row being sown with dried seed, and the other with seed which had just been taken from the refrigerator. The third row was sown with unvernalized seed. The heads in the two rows sown with vernalized seed emerged two days earlier than those in the control row, but all three rows ripened together.

When the plants in the three rows sown with infected seed had headed out, counts were made of the number of smutted and healthy plants in each row. The results of the counts are summarized in Table 1.

TABLE 1.—THE EFFECT OF VERNALIZATION ON THE SURVIVAL OF LOOSE SMUT IN ARTIFICIALLY-INOCULATED SEED OF MARQUIS WHEAT

| Treatment | No. seeds | Total plants | Smutted plants | Per cent smut |
|---|---|---|---|---|
| Vernalized | 150 | 95 | 61 | 64 |
| Vernalized and dried | 150 | 51 | 23 | 45 |
| Not vernalized | 150 | 111 | 64 | 58 |

Vernalization resulted in a considerable reduction in the number of plants per row as compared with the unvernalized control. The reduction was greatest in the row sown with dried vernalized seed. Judging by the percentages of smutted plants, the process of vernalization had no observable effect on the loose smut mycelium. The seed which was dried after vernalization produced relatively fewer smutted plants, but this was possibly due to the smaller number of plants on which the smut count was based.

## SUMMARY

1. The effect of environmental conditions on the development of loose smut of wheat and barley is discussed, and the literature on this subject is reviewed.

2. The incidence of loose smut in artificially-inoculated seed of Marquis wheat was not appreciably affected by vernalization.

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## Résumé

**Effet de la vernalisation sur la fréquence du charbon nu du blé, par W. F. Hanna, Laboratoire fédéral de la rouille, Winnipeg, Man.**

L'effet des conditions environnantes sur le développement du charbon nu du blé et de l'orge est étudié, et les ouvrages traitant de ce sujet sont passés en revue. La fréquence du charbon nu dans la semence de blé Marquis infecté d'une manière artificielle, n'a pas été affectée d'une façon appréciable par la vernalisation.